Liver transplantation for the treatment of hepatocellular carcinoma

TOSO, Christian

Abstract
Liver transplantation is the best treatment option for selected patients with non-resectable hepatocellular carcinoma (HCC). In many centers, patient selection is currently performed according to Milan criteria and allowing transplantation for patients with a single HCC ≤5cm or up to 3 HCCs ≤3cm. The present thesis demonstrates that patients with more advanced HCCs, with total tumor volume ≤115cm³ and alpha-fetoprotein ≤400ng/ml, can also benefit from liver transplantation and have similarly good post-transplant outcomes. Another way of allowing transplantation to patients with more advanced HCC, is to downstage HCCs prior to listing. This option is also discussed. Adjuvant post-transplant management should be performed with the use of sirolimus, an immunosuppression drug with anti-cancer properties. This drug has an appropriate side-effect profile and improves post transplantation survival in patients with HCC. Such strategies will help including more patients for transplantation, with maintained good post-transplant survivals.

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Liver transplantation for the treatment of hepatocellular carcinoma

Christian Toso MD, PhD

Services de chirurgie viscérale et transplantation
Département de chirurgie
Hôpitaux Universitaires de Genève
Faculté de Médecine
Université de Genève

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Abstract

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The present thesis demonstrates that patients with more advanced HCCs, with total tumor volume ≤115cm$^3$ and alpha-fetoprotein ≤400ng/ml, can also benefit from liver transplantation and have similarly good post-transplant outcomes.

Another way of allowing transplantation to patients with more advanced HCC, is to downstage HCCs prior to listing. This option is also discussed.

Adjuvant post-transplant management should be performed with the use of sirolimus, an immunosuppression drug with anti-cancer properties. This drug has an appropriate side-effect profile and improves post transplantation survival in patients with HCC.

Such strategies will help including more patients for transplantation, with maintained good post-transplant survivals.
Acknowledgements

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A special thank goes to my clinical colleagues in Geneva, including Prof. Thierry Berney, Drs. Emile Giostra, Pietro Majno and Isabelle Morard, for the friendly and professional daily interactions and the fruitful discussions and collaborations on multiple scientific projects.

Finally, I will always remain deeply grateful to my parents, Alain and Colette Toso, and to Seema for their generous love and limitless support.
1. Introduction
Epidemiology and screening of hepatocellular carcinoma

Hepatocellular carcinoma results in between 250,000 and one million deaths globally per annum. It is the third most frequent cause of cancer-related death worldwide, with a steady rise in incidence in most developed countries (1). The rate of HCC varies a lot according to geographic locations, reflecting the regional variations in exposure to risk factors such as hepatitis B and C viruses (HCV, HBV) and aflatoxin (2). In Switzerland, the incidence of HCC is 10.2 /100,000 per year in males and 1.5 /100,000 per year in females. This represents about 600 new cases per year, corresponding to 1.7% of all new cases of cancer (www.liguecancer.ch). The Swiss incidence is relatively high compared to other European countries, probably linked to the incidence of HCV (Figure 1.1).

Figure 1.1
These European incidence rates remain however significantly lower than those observed in Asia and Africa, where the rates of HBV and HCV are higher. To illustrate, the incidence of HCC per 100,000 male individuals per year are 34.4 in China, 19.9 in Philippines, 25.8 in Nagasaki, 64.6 in Zimbabwe and 113 in Mozambique.

In most Western countries, the incidence of HCV-related HCC is expected to plateau in about a decade, reflecting the high HCV infection rates between 1960 and 1990 and the 20-30 year lag time between infection, and the development of cirrhosis and HCC (3). It is however difficult to predict the behaviour of the overall rate of HCC, because of the rising incidence of non-alcoholic fatty liver disease (NAFLD), which is also a high risk factor for HCC (4).

On a public health stand-point, screening of individuals at risk is the most efficient way to decrease mortality. As such, it is recommended that patients at risk undergo bi-annual liver ultrasound with or without a dosage of alpha-fetoprotein (AFP). According to the AASLD guidelines, this should be offered to patients with cirrhosis or to HBV carriers ≥40 years for males and ≥45 years for females or with a family history of HCC (www.aasld.org/practiceguidelines). This strategy should be further promoted, as most HCCs are still diagnosed late in their course, preventing any potentially curative treatment (5).
Clinical presentation and diagnosis of HCC

The diagnosis of HCC is difficult. Although some HCC patients can present hypoglycemia (high tumor metabolic demand or local secretion of insulin-like growth factor II), erythrocytosis (tumor secretion of erythropoietin), hypercalcemia (metastasis or secretion of PTH-related protein) and diarrhea, most do not develop any symptom specific to HCC (beside those linked to the underlying cirrhosis)(6-9). Some can also have non-specific (thus usually not helping for diagnosis) skin lesions, including dermatomyositis, pemphigus foliaceus, sudden and multiple seborrheic keratosis (Leser-Trelat sign), pityriasis and porphyria cutanea tarda (10). Due to the lack of specific symptoms, many HCCs are discovered late or only thanks to the ultrasound and AFP screening.

The discovery of a liver nodule in a cirrhotic liver should raise suspicion of HCC. While the association of a clear nodule with an elevated AFP (≥100 ng/ml) is nearly pathognomonic of HCC (specificity of 99%), the exact characterization of many nodule remains challenging, especially for small lesions (≤1 cm) (11, 12). In many patients, only the combination of multiple tests can lead to diagnosis. While AFP is the tumor marker most used, its positive predictive value is low, and several other markers have been tested (Table 1.1)(13, 14). At the present time, none of them reached a similar acceptance as AFP.
- Alpha fetoprotein
- Des-gamma-carboxy prothrombin (also known as Prothrombin produced by vitamin K absence or antagonism II)
- Tumor-associated isoenzymes of gammaglutamyl
- Transforming growth factor-β1
- Circulating intercellular adhesion molecule-1
- Alpha-L-fucosidase
- Hepatocyte growth factor
- Golgo protein-73
- Glypican-3 (cell-surface heparan proteoglycan)

Table 1.1: markers associated with HCC

On an imaging point of view, ultrasound, CT and MRI are the most often used tests. Ultrasound is widely available, non invasive and commonly used as screening test. CT and MRI are used as second line tests in order to better characterize the initial US discovery. According to a recent systematic review, MRI appears to have the best sensitivity and specificity combination (Table 1.2).

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>60 (44-76)</td>
<td>97 (95-98)</td>
</tr>
<tr>
<td>CT</td>
<td>68 (55-80)</td>
<td>93 (89-96)</td>
</tr>
<tr>
<td>MRI</td>
<td>81 (70-91)</td>
<td>95 (77-93)</td>
</tr>
</tbody>
</table>

Table 1.2: Accuracy of US, CT and MRI for the diagnosis of HCC (15)

Overall, the diagnostic strategy of HCC is adapted according to tumor size (www.aasld.org/practiceguidelines). Lesions smaller than 1 cm in diameter
should be monitored with repeat imaging at 3-4 months, and further assessed in case of growth. Larger nodules should be assessed by one or multiple imaging modalities, according to tumor size and AFP (in case of a large typical HCC on radiology, only one imaging is enough, especially if AFP is increased). Of note, a typical HCC is hypervascular in the arterial phase and washes out in the portal/venous phase. Biopsy should only be used in case of persistently unclear diagnosis, because of the risk of cancer seeding with the biopsy needle.
Treatment options for HCC

The treatment of HCC is currently an extremely active field of investigation, as testified by several conferences, including a US national conference in 2009 (16), a European Association for the Study of Liver (EASL) special conference in June 2010 and an international consensus conference on liver transplantation for HCC organized by Dr. Pierre-Alain Clavien in Zurich, Switzerland in December 2010. At the present time, most patients still present very advanced HCCs at the time of diagnosis, and only palliative management can be offered. This includes local HCC treatment with transarterial chemoembolisation (TACE), radio-frequency ablation (RFA) and ethanol injection, and/or systemic drugs like sorafenib. Palliative treatment leads to only poor results and high fatality rates, as reflected by the number of annual deaths for HCC, which is very close to the incidence of newly diagnosed cases (17). Such management will not be further explored in this thesis, which is mainly investigating the use of transplantation for the treatment of HCC.

Curative treatments of HCC include resection and transplantation. Resection will be primarily offered to patients with preserved liver function (Child A) and localized HCC. Conversely, transplantation can be potentially performed in any HCC patient. It should however only be offered in individuals with good expected post-transplant outcomes and the most commonly used selection criteria are those developed by Dr. Vincenzo Mazzaferro in 1996 in Milan (18). In the absence of metastasis or major vessel invasion, they allow transplantation for
patients with a single HCC ≤5 cm or up to 3 HCCs each ≤3 cm. With the application of such criteria, 5-year post-transplant survivals >70% can be expected (19).

Allowing transplantation in patients with more advanced HCCs

Recently, several studies have demonstrated that the Milan criteria are too restrictive and that favorable outcomes can be achieved following more liberal selection policies (19, 20). Dr. Francis Yao and the group at the University of California, San Francisco (UCSF), were the first to propose expanded criteria (21). While validated by other centers (22-25), the UCSF criteria have failed to gain unanimous recognition, possibly because they exclude patients with more than three HCC’s (even when of small size and with expected favorable outcomes) and include patients with large tumors (up to 6.5 cm in diameter), which have been associated with decreased post-transplant outcomes (26-28). In addition, a large multicenter study has suggested decreased outcomes in patients between Milan and UCSF criteria (23). As a consequence, many other groups have proposed alternative morphological scores to select transplant candidates (19, 29-40). The backbone of most of these scores is a combination of HCC size and number, with or without the addition of alpha-fetoprotein (AFP) or tumor grade.

The acceptance of new expanded selection criteria should be based on the observation that the newly recruited patients (beyond Milan, but within the new
score) have stable and acceptable intent-to-treat survivals compared to those within Milan criteria.

The present thesis intends to better define which patients can benefit from liver transplantation with good outcomes, and how far beyond Milan selection should go. This will be performed looking at the total tumor volume (TTV), alone or in combination with AFP (Chapters 2 and 3). These assessments will be based on patient populations from Edmonton, Denver and Toronto in one study and on the Scientific Registry for Transplant Recipients (SRTR), a large US transplant registry, in the other.

When considering expanding selection criteria, it is important for Centers and policy agencies to be able to predict the need for resources linked to an expansion of criteria, as this has the potential to impact the work load as well as the need for alternative sources of organs, including live donors or donors after cardiac death. The study in Chapter 3, based on the Alberta Cancer Registry, provides the estimated impact of various previously proposed expanded criteria on the number of newly recruited transplant candidates.

Beside expanding criteria, patients with more advanced HCCs (beyond classical Milan) can also be considered for downstaging to currently accepted criteria. This option will be discussed in Chapter 4, underlining its benefits and limitations, and proposing guide-lines based on the currently available literature.

*Improving post-transplant adjuvant cancer management*
The use of de novo sirolimus after liver transplantation, has remained limited (41-45), mainly because of a “Black Box” warning from the US Food and Drug Administration. This followed two multicenter phase II/III trials (46, 47, www.fda.org), suggesting that sirolimus was associated with a trend towards increased rates of hepatic artery thrombosis within the first 3 weeks post-transplant. Although the trend has not been confirmed by subsequent studies (41, 42, 44, 45, 48), the warning remains.

At the time, sirolimus-based immunosuppression has been associated with a reduced risk of de novo malignancy after all types of transplantation (49). This activity against cancer was suggested in the mid 1980’s (50) and has been further supported by more recent data. Sirolimus can prevent angiogenesis by interfering with VEGF-mediated pathways in endothelial cells, thus limiting the growth of tumors (51). It impacts on established tumor vessels, by inducing extensive microthrombi (52) and can inhibit the growth of human hepatoma cells in vitro (53).

While these data suggest clear anti-cancer properties, it remains to be determined whether sirolimus can reduce the risk of post-transplant recurrence in patients with HCC. While no randomized study is available to date, some institutions, have demonstrated the suitability of sirolimus used from the time of transplantation for HCC (42, 54, 55). These preliminary studies have reported on limited numbers of patients and relatively short follow-ups.

Chapters 6 and 7 will assess the impact of sirolimus on post-transplant HCC recurrence and patient survival rates. One study is based on the unique recipient
population in Edmonton, where this drug has been used since 1996. It investigates the long-term outcomes and reports on its side-effect profile. The other study is again based on the SRTR registry, and looks at the impact of sirolimus on post-transplant survival in a large patient population. Chapter 8 will subsequently explore the use of sirolimus in case of live donor liver transplantation.

All these data will be discussed in Chapter 9, suggesting a more liberal inclusion of HCC patients for transplantation than those offered by Milan criteria. This can be done by expanding current criteria and/or downstaging. The use of sirolimus in case of transplantation for HCC will be promoted, thus challenging the current FDA black box warning.
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Liver transplantation using sirolimus and minimal corticoids (3-day taper). 7 ed. 2001. 343-351.
2. Patient selection based on Total Tumor Volume

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by Christian Toso, James Trotter, Alice Wei, David L. Bigam, Shimul Shah,
Joshua Lancaster, David R. Grant, Paul D. Greig, A.M. James Shapiro, Norman
M. Kneteman
Abstract

Criteria for the selection of candidates for liver transplantation in the presence of hepatocellular carcinoma (HCC) should accurately predict posttransplant recurrence while not excluding excessive numbers of patients from candidacy. Existing criteria are challenged by the limited accuracy of radiological assessment. The total tumor volume (TTV) was calculated by the addition of the volume of each individual tumor. A preliminary analysis was carried out on HCC patient data from the Alberta Liver Transplant Program (52 patients) and then validated on the populations of the Universities of Toronto and Colorado programs (154 and 82 patients). A TTV cutoff of 115 cm$^3$ was chosen on the basis of the risk of recurrence with use of a receiver operating characteristic curve. Radiology correlated more closely to pathology with TTV than with Milan and University of California at San Francisco (UCSF) criteria (91% versus 69% and 75% of patients, $P \leq 0.0001$). Although more patients met qualifying criteria for transplant with TTV (28%-53% more than Milan and 16%-26% more than UCSF), no deterioration of outcome was demonstrated in an analysis of patients within TTV $\leq 115$ cm$^3$ in comparison with those meeting Milan or UCSF classifications at all institutions. Patients with TTV $> 115$ cm$^3$ experienced more recurrences and lower patient survival in the Alberta and Colorado series ($P \leq 0.05$). When TTV with a cutoff of 115 cm$^3$ is used for candidate selection, the accuracy of pretransplant radiological assessment is enhanced, with posttransplant outcomes not different from those achieved with Milan and UCSF classifications despite a more inclusive patient population.
Introduction

Liver transplantation is a rational therapeutic option in selected patients with hepatocellular carcinoma (HCC) and underlying cirrhosis. Various candidacy evaluation systems are currently available to assess patients with HCC. The Milan criteria were first described in 1996, and include patients with a single tumor up to 5 cm in diameter or up to 3 tumors none larger than 3 cm (19). This classification has been widely used, but has been felt by many, including by Mazzafero et al. in recent reports (20), to be too restrictive, denying access to liver transplantation to a substantial number of patients who may expect benefit. A more extended set of inclusion criteria was subsequently proposed by the UCSF group (21). The latter includes patients with a single tumor up to 6.5 cm diameter or ≤3 tumors, none larger than 4.5 cm and with a cumulative diameter up to 8 cm. While these selection criteria were originally designed on single-centre patient populations, they have now been applied in several subsequent reports (22-24). Unfortunately the clinical applicability of both of these systems is challenged by the low accuracy of the pretransplant radiological assessment, on which transplant listing decisions are based. Using these classifications, radiology matches the pathology in only 40 to 60% of patients (24-26). There is thus a need for a pre-transplant selection classification achieving transplant outcomes at least equivalent to the Milan and UCSF criteria, but with improved pretransplant radiological accuracy and with candidacy that does not exclude patients with expectation of good outcome. Such a classification could improve today’s practice and could in the future be combined with other tests currently
under development, including assessment of tumor cell ploidy or of gene expression from tumor biopsy or blood (27).

This three-center study investigated the Total Tumor Volume (TTV) as a pre-transplant selection tool, defined a cut-off value and compared it to other staging systems to predict transplant outcome. It also assessed the ability of these classifications, when applied to radiological studies carried out before transplantation, to predict both the tumor staging on explant pathology and ultimate tumor free survival.

**Patients and methods**

*Patient inclusion*

Only liver recipients documented to have HCC pre-transplant were included in the study. Patients with incidental HCC discovered on the explanted liver were excluded. They were transplanted at the University of Alberta, Edmonton, Canada (Alberta, from December 1996 to February 2006), at the University of Toronto, Toronto, Canada (Toronto, from January 1996 to January 2006) and at the University of Colorado Health Sciences Center, Denver, USA (Colorado, January 2002 to January 2007). This study was approved by all institutional ethical review board committees.

All institutions used extended inclusion criteria. Selection criteria for transplantation at the University of Alberta were a single HCC up to 7.5 cm, or multiple tumors (without number restriction) up to 5 cm in diameter. When an HCC was larger than 5 cm in diameter, a biopsy was obtained to rule out poorly
differentiated tumor; having a tumor that was both larger than 5 cm and poorly
differentiated would disqualify the patient from transplant. At the University of
Toronto, Milan selection criteria were followed till September 2004. After this
date, no size and number restrictions were used, but patients were excluded if
they had large (>5 cm) tumors that were also poorly differentiated on liver biopsy.
At the University of Colorado, Milan criteria were followed, except for living-
related donor transplants, who were included patient by patient, without size and
number restrictions. In all three centers, patients with extra-hepatic disease or
major vascular invasion on imaging were excluded from candidacy throughout
the study time period.

**Immunosuppression**

In Alberta, immunosuppression was sirolimus-based (target trough range of 12-
15 μg/L) with low-dose calcineurin inhibitors. Early patients also received
corticosteroids for 3 months; induction therapy with daclizumab replaced steroids
for the last 35 patients.

In Toronto, immunosuppression included calcineurin inhibitors and steroids. HCV
patients were put on cyclosporin, non-HCV patients on tacrolimus. Living-related
donor recipients received induction therapy with thymoglobulin or Simulect.

In Colorado, immunosuppression was sirolimus-based with calcineurin inhibitors
and 3-day corticosteroids taper till 2003. It subsequently included tacrolimus,
mycophenolate mofetil and short-term (usually less than one week)
corticosteroids.
Outcome assessment and statistical analysis

Data were collected prospectively in an electronic database (OTTR, Hickman-Kenyon Systems, Omaha, NE) and were analyzed retrospectively. Median follow-up was 25 [1-112] months.

Radiological staging was assessed from reports of contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI) obtained closest to the time of transplant or prior to pre-transplant ablative therapy. A lesion was considered an HCC, when it demonstrated an arterial enhancement with wash-out on delayed images, or when significant increase in size was documented. A stable, non-enhancing small lesion was not considered as HCC. In cases of pre-transplant tumor treatment, pathological estimate of tumor volume included the size of the remaining HCC together with the size of necrotic tumor. The Total Tumor Volume was calculated as the sum of the volumes of all tumors \((4/3)\pi r^3\), \(r\): maximum radius of each HCC). Patient survival was defined as survival with or without HCC recurrence. Patients were classified according to Milan and UCSF criteria as previously described (1,3).

Analysis was initially performed on the Alberta Liver Transplant Program patient population only (52 patients), in order to assess the Total Tumor Volume and to select a cut-off value. The cut-off was determined according to the risk of HCC recurrence on a Receiver Operating Characteristic (ROC) curve. It was selected to achieve the best sensitivity/specificity combination.
Pretransplant radiological staging was next compared to the pathological staging. The radiological accuracy was determined as the rate of patients correctly classified by radiology according to Milan, UCSF and TTV scores. Patients with radiology up-staging pathology were patients beyond the specific score on radiology, but within on pathology. Patients with radiology down-staging pathology were patients within the specific score on radiology, but beyond on pathology. Survival was analyzed by the Kaplan-Meier method and differences between groups were further tested by univariate analysis using the log-rank test. Multivariate analysis using a Cox proportional hazards model was used between the prognostic factors reaching at least 0.1 on univariate analysis. These analyses were conducted on the Alberta patient population. As a validation process, the model was used to predict recurrence and survival on two independent populations of patients from Toronto and Colorado. Analyses were also performed using both pathological and radiological assessments. Further tests were performed using Chi-square or Fisher test for categorical variables and t test for continuous variables. P values less than 0.05 were considered significant. Calculations used Stata (College Station, Texas, USA) and Statistica (Statsoft, Berikon, Switzerland) software.
Results

Patient characteristics

All studied patient populations demonstrated similar characteristics, with more males and median ages of 54, 57 and 54 years (Table 2.1). Primary underlying liver diseases were most often related to viral hepatitis (Table 2.1). Roughly half of the patients in each program had pre-transplant treatment to control tumor growth. There were differences in practice between the three institutions with use of alcohol injection more frequent in Alberta, radiofrequency ablation in Toronto and transarterial chemoembolization (TACE) in Colorado (Table 2.1).

<table>
<thead>
<tr>
<th></th>
<th>Alberta (%)</th>
<th>Toronto (%)</th>
<th>Colorado (%)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Patients (number)</td>
<td>52</td>
<td>154</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Median age (years)</td>
<td>54 (range 37-67)</td>
<td>57 (range 28-70)</td>
<td>54 (range 33-65)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender</td>
<td>male:45</td>
<td>female:21/1</td>
<td>female:11/</td>
<td>NS</td>
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<tr>
<td>Cause of liver disease (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV (±alcohol, ±HBV)</td>
<td>23 (44)</td>
<td>83 (54)</td>
<td>58 (71)</td>
<td>*</td>
</tr>
<tr>
<td>HBV</td>
<td>16 (31)</td>
<td>29 (19)</td>
<td>7 (9)</td>
<td>*</td>
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<tr>
<td>Alcohol</td>
<td>4 (7.5)</td>
<td>25 (16)</td>
<td>7 (9)</td>
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</tr>
<tr>
<td>Cryptogenic</td>
<td>3 (5.5)</td>
<td>6 (3.5)</td>
<td>2 (2)</td>
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<td>0</td>
<td>5 (3)</td>
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<td>0</td>
<td>3 (2)</td>
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<td>NS</td>
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<td>Primary biliary cirrhosis</td>
<td>3 (6)</td>
<td>1 (0.5)</td>
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<td>1 (0.5)</td>
<td>1 (1)</td>
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<tr>
<td>Primary sclerosing cholangitis</td>
<td>1 (2)</td>
<td>0</td>
<td>2 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>Various</td>
<td>0</td>
<td>1 (0.5)</td>
<td>3 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>Pre-transplant treatment (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol injection</td>
<td>13 (25)</td>
<td>14 (9)</td>
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<td>***</td>
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<tr>
<td>TACE</td>
<td>5 (9.5)</td>
<td>6 (4)</td>
<td>41 (51)</td>
<td>*</td>
</tr>
<tr>
<td>Surgical resection</td>
<td>3 (6)</td>
<td>7 (5)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Radiofrequency ablation</td>
<td>3 (6)</td>
<td>42 (27)</td>
<td>1 (1)</td>
<td>****</td>
</tr>
<tr>
<td>None</td>
<td>28 (53.5)</td>
<td>85 (55)</td>
<td>39 (48)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Denver vs Alberta or Toronto: p≤0.05
** Denver vs Alberta: p≤0.05
*** Alberta vs Toronto or Denver: p<0.01
**** Toronto vs Alberta or Denver: p <0.01

HCV: hepatitis C virus infection, HBV: hepatitis B virus infection

Table 2.1: Patient and tumor characteristics
Tumor recurrence and survival

Seven patients experienced a tumor recurrence in the Alberta series (13%), 15 at Toronto (10%) and 10 in Colorado (12%, p: NS). The median time between transplant and diagnosis of recurrence was 11 months (1.3-51.7). Twelve, 28 and 13 patients died during the post-transplant observation period in Alberta, Toronto and Colorado (p: NS), among them 6, 11 and 10 had experienced a recurrence. Patient survivals were similar at all three institutions. One and five year survivals were 87 and 75% in Alberta; 90 and 72% in Toronto and 90 and 80% in Colorado (p: NS).

Selection of a Total Tumor Volume cut-off value

The selection of a TTV cut-off was performed on the Alberta study population data only. According to pathology, patients with higher calculated TTV were more at risk of recurrence (Figure 2.1). ROC analysis identified an optimal cut-off value of 115 cm$^3$ to differentiate HCC-free patients from those with recurrence (Figure 2.2A). This could be achieved with good diagnostic accuracy (Area Under Curve, AUC: 0.8). With this cut-off, sensitivity was 71%, specificity 84%, positive predictive value (PPV) 42% and negative predictive value (NPV) 95%. For comparison, application of Milan and UCSF criteria to predict recurrence in the same dataset gave sensitivities of 86 and 57%, specificities of 47 and 60%, PPV of 20 and 18% and NPV of 95 and 90%.
A TTV ROC assessment was also performed based on radiology (Figure 2.2B). The AUC was 0.6. The same cut-off of 115 cm$^3$ was found to be appropriate and achieved a sensitivity of 29%, a specificity of 98%, a PPV of 67% and a NPV of 90%.
Figure 2.1: Distribution of patients with and without HCC recurrence in Alberta according to Total Tumor Volume, based on pathological staging.
Figure 2.2: ROC curve assessments of the pathological (A) and radiological (B) Total Tumor Volume scores to predict post-transplant HCC recurrence. Areas Under Curves (AUC) were 0.8 and 0.6 respectively. A cut-off of $115\text{cm}^3$ was selected (*). It provided a sensitivity and specificity of 71% and 84% with pathology (A) and 29% and 98% with radiology (B). Diagonal segments were produced by ties.
Pre-transplant radiological selection accuracy

The accuracy of pre-transplant imaging was similar at all three institutions. Overall, radiological staging better matched explant pathology with application of the TTV criteria when compared to Milan and UCSF classifications (91 vs. 69 and 75% of patients, $p \leq 0.0001$, Table 2.2). Errors of classification were most often due to understaging on radiology when compared to pathology. In keeping with previous reports, the absolute number of tumors was frequently assessed incorrectly with radiology; this was the case in 60, 47 and 59% of patients in Alberta (31/52), Toronto (71/154) and Colorado (48/82) respectively (this rate included any patient with a HCC number mistake, whether this impacted on Milan-UCSF staging or not).
Table 2.2: Performance of pre-transplant radiological assessment compared to pathology

<table>
<thead>
<tr>
<th></th>
<th>Milan (%)</th>
<th>UCSF (%)</th>
<th>TTV* (%)</th>
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* Total Tumor Volume
NS: not significant
Analyses of variables predicting survival

Using univariate analyses, several pathology-based variables predicted survival and were summarized in Table 2.3. On multivariate Cox analysis, TTV was the only predicting factor in both Alberta (p=0.01, Table 2.4) and Colorado (p ≤0.0001). In Toronto, AFP level (p ≤0.05) and macrovascular invasion (p ≤0.0001) were significant.

Using radiology-based classifications, we further performed the same analysis looking at variables that might predict survival. Median time between images and transplantation was 2 (0.03-13) months. TTV tended to predict patient survival in Alberta (p ≤0.09) and in Colorado (p ≤0.05). Beside tumor size (p ≤0.01 in Colorado), none of the other variables reached statistical significance.

When combining all centers and using both pathology- and radiology-based staging, patients beyond Milan, but within TTV ≤115 cm^3 had similar survivals than those within Milan (Figures 2.3A, 2.4A). On the contrary, patients with TTV >115 cm^3 demonstrated lower survival than those within TTV ≤115 cm^3, using pathology (5-year: 47% vs 79%, p ≤0.001) and radiology staging (5-year: 53% vs 76%, p=0.1).
Table 3: Univariate analysis of factors impacting on patient survival from pathological staging

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<th>Toronto</th>
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<th>Patients (%)</th>
<th>Five-year patient survival (%)</th>
<th>Colorado</th>
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Milan criteria as previously defined by Mazzaferro et al. (1)
UCSF criteria as previously defined by Yao et al. (2)
Grade I: well differentiated, grade II: moderately differentiated, grade III: poorly differentiated
NS: not significant

Table 2.3: Univariate analysis of factors impacting on patient survival from pathological staging
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<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
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<td>1.6-4.9</td>
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<td>Alpha feto-protein level (≤10 vs &gt;10)</td>
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<td>1.1-10.2</td>
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<tr>
<td>Colorado</td>
<td>Total Tumor Volume (≤115 vs &gt;115)</td>
<td>13.9</td>
<td>4.3-44.9</td>
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</table>

Table 2.4: Step by step multivariate Cox analysis using pathology staging (significant variables)
Table 2.3: Pathology-based staging: cumulative survival (A, log rank: p=0.2) and cumulative risk of HCC recurrence (B, p=0.1) for all patients grouped as within Milan or beyond Milan-within TTV (≤115 cm³). Numbers of patients at risk are reported under each specific time point.
Figure 2.4: Radiology-based staging: cumulative survival (A, log rank: $p=0.3$) and cumulative risk of HCC recurrence (B, $p=0.3$) for all patients grouped as within Milan or beyond Milan-within TTV ($\leq 115 \text{ cm}^3$). Numbers of patients at risk are reported under each specific time point.
Analyses of variables predicting tumor recurrence

Using both pathological and radiological staging, the overall risk of HCC recurrence remained stable at all institutions, for patients within TTV classification, but increased for patients with a TTV exceeding 115 cm$^3$:

When using pathological staging, no significant difference could be observed (p=NS between all conditions) between patient groups defined as within Milan (1/22, 5%; 6/85, 7% and 2/50, 4% in Alberta, Toronto and Colorado), beyond Milan-within UCSF (2/8, 25%; 2/21, 9.5% and 0/7, 0%) and beyond UCSF-within TTV (0/12, 0%; 5/35, 14% and 1/11, 9%) classifications. However, the risk increased when patients had a TTV exceeding 115 cm$^3$, reaching 50% in Alberta, 15% in Toronto and 50% in Colorado. This was significantly higher compared to recurrence rates for patients who were within Milan, UCSF and TTV (≤115 cm$^3$) criteria in Alberta (p ≤0.01, ≤0.05 and ≤0.001) and in Colorado (p ≤0.001, ≤0.005 and ≤0.00001).

When using radiological staging, and except for an isolated increase in Colorado patients beyond Milan-within UCSF, the risk of HCC recurrence also remained stable when using radiology-based Milan (4/32, 12.5%; 8/99, 8% and 6/61, 10% in Alberta, Toronto and Colorado), beyond Milan-within UCSF (1/7, 14%; 5/29, 17% and 2/5, 40%) and beyond UCSF-within TTV (0/10, 0%; 2/21, 9.5% and 0/12, 0%) staging systems. In Alberta, two of three patients with TTV higher than 115 cm$^3$ on radiology, experienced a recurrence. This rate was significantly higher than the 10.2% in patients with TTV smaller than 115 cm$^3$ (p ≤0.05).

Similarly in Colorado, two of four patients with TTV higher than 115 cm$^3$ on
radiology, experienced a recurrence ($p \leq 0.05$ vs. patients with $TTV$ smaller than $115 \text{ cm}^3$).

When assessing all groups together, and using both pathology and radiology-based staging, the cumulative risk of HCC recurrence (assessed by Kaplan Meier) was similar in patients beyond Milan, but within $TTV \leq 115 \text{ cm}^3$ compared to those within Milan (Figures 2.3B, 2.4B). On the contrary, patients with $TTV > 115 \text{ cm}^3$ demonstrated a higher cumulative risk of HCC recurrence than those within $TTV \leq 115 \text{ cm}^3$, using pathology (5-year: 58% vs. 13%, $p \leq 0.001$) and radiology staging (5-year: 39% vs. 17%, $p \leq 0.01$).

**Number of patients included for transplantation**

More patients would have been selected for transplantation with $TTV$, compared to Milan and UCSF. Using pretransplant radiology, 49, 147 and 78 patients would have qualified for transplantation with $TTV$; 32, 99 and 61 with Milan and 39, 127, 66 with UCSF classifications, in Alberta, Colorado and Toronto. The number of transplant candidate increased between 28 and 53% compared to Milan and between 16% and 26% compared to UCSF.
Discussion

This study suggests that Total Tumor Volume may be a useful tool to assess patients with HCC for consideration of liver transplant. Using this staging system, with a cut-off of 115 cm$^3$, the accuracy of pretransplant radiology can be enhanced, with post-transplant outcomes not different from those achieved with Milan and UCSF classifications, despite inclusion of more patients for transplant.

The present study determined, like others (24, 28, 29), that macrovascular tumor invasion is strongly associated with post-transplant tumor recurrence. The discussed TTV classification should therefore only be considered in patients without major vascular involvement by tumor on pretransplant imaging. In addition, both lymph node and other extrahepatic metastases remain as contraindications for transplantation.

The use of current selection criteria for HCC patients has been challenged by the low accuracy of staging by pre-transplant radiology when compared to explant pathology, usually reaching only 40 to 60% (24-26). In the current report, accuracy was higher than in previous studies, reaching 60 to 70% for Milan and 70 to 80% for UCSF classifications. However, these outcomes were significantly poorer than those achieved with the TTV system, where 85 to 95% accuracy of pre-transplant radiological diagnostic staging was achieved.

The improved accuracy is substantially related to the fact that TTV gives more power to the largest tumors, as the calculated volume increases much faster than the related diameter, the mathematical formula for volume being $(4/3)\pi r^3$. As a
consequence, the likelihood of correct radiological staging is increased, because larger tumors can be more accurately assessed than smaller ones, and larger tumors impact much more strongly on the calculation of TTV. Stiropoulos et al reported sensitivities of radiology to detect HCC seen on pathology of 0% for tumors smaller than 1 cm, 21% between 1 and 2 cm, 77.5% between 2 and 5 cm and 100% for tumor larger than 5 cm (25). False positive tumors also tend to involve smaller lesions (30, 31).

As a consequence of the difficulty in assessment of small HCC, the number of tumors had been correctly determined in only 40 to 53% of patients. The TTV staging system does not exclude patients based solely on the number of lesions. To illustrate: a patient with one tumor of 4 or 5 cm diameter and 3 tumors of 0.5 cm diameter would be ruled ineligible for liver transplant under both the Milan and the UCSF criteria. Such a patient would be eligible under the TTV criteria as the 3 small tumors have little impact on overall tumor volume (TTV = 33.5 and 65.4 cm$^3$ respectively). Our data also suggest such a patient could be expected to have a very good long term tumor free survival, and as such would be poorly served by current systems of candidacy evaluations. Our findings that the number of tumors per se does not accurately predict tumor recurrence and survival after liver transplant is consistent with other studies (19, 23, 24, 29, 32).

Of note, about half of the patient included in our study received a pre-transplant HCC treatment, which, per nature, has the potential to impact on the size of the lesion. As such, some discrepancy between radiology and pathology may be
related to treatment. Of importance however, this would have impacted similarly on the assessment of the three scores (Milan, UCSF, TTV).

TTV was selected for evaluation as a potential useful predictor of post-transplant outcome, based on the observation that the occurrence of micro-satellite HCC metastasis increases exponentially with tumor size, matching tumor volume (33). Several groups further reported that patients with large tumors have diminished tumor-free survivals (21, 28, 32, 34). Survival after liver resection of colorectal metastasis can also be predicted by the tumor volume (35).

We first determined a cut-off value from the patient population of the Alberta Liver Transplant Program, according to the risk of recurrence and using a ROC curve. Both pathological and radiological assessments provided a total volume cut-off of 115 cm$^3$. By choosing a higher cut-off, more patients would have been selected for transplant, but the risk of recurrence would have increased significantly, decreasing the specificity of the test. In contrast, were a lower cut-off value utilized, several patients with a high likelihood of good long term outcome would have been excluded from transplant, decreasing the sensitivity of the test. A cut-off of 115 cm$^3$ corresponds to a single tumor of approximately 6 cm in maximum diameter or three lesions of approximately 4.2 cm.

As a validation process, assessments were performed separately on the three patient populations. Of note, all three teams have used extended selection criteria, including patients beyond Milan, UCSF and TTV classifications. Analyses were done using both pathological and radiological staging.
TTV accurately predicted recurrence and patient survival in both Alberta and Colorado series. This was not the case in the Toronto series. This may be linked to the low number of patients included with TTV >115 cm$^3$ in the Toronto series (only patients within Milan criteria were accepted in Toronto before 2004), and possibly also to the fact that patients with poorly differentiated large tumors were excluded from transplant candidacy (they would be predicted to have a higher risk of recurrence). As such, the Toronto validation was probably underpowered. Along the same line and while large poorly differentiated tumors were also excluded in Alberta, the reader must be aware of this specificity of the study and further validation in a non pre-selected group appears of interest. Of note, one would expect higher rates of recurrence, especially in the groups including large tumors (patients with TTV exceeding 115 cm$^3$).

Importantly, when respecting a TTV cut-off of 115 cm$^3$, we observed overall similar rates of recurrence and patient survival with TTV, Milan and UCSF classifications at all three institutions despite a more inclusive patient population with the TTV system. Recurrence rates in all three centers remained around or lower than 10% with both radiological and pathological staging and are in accordance with previously published results (19, 22, 23, 29, 34, 36-38).

A point of caution has to be raised as the TTV cut-off was determined based on the Alberta population, which includes only seven recurrences. An error in one or two cases could potentially have modified the assessment of the cut-off. However, the selected cut-off in the Alberta population ultimately proved to be the ideal cut-off in the Colorado population as well. Of interest, TTV is a
continuous scale, and the cut-off could be modified by addition of further variables as well as to centers’ local policies and results, with application of higher or lower levels of expected recurrence.

While prospective and more consistent trials are required, our results suggest that TTV, with a cut-off of 115 cm$^3$, may be a useful tool for selection of patients with HCC for transplant candidacy. TTV would allow the transplantation of more patients than with Milan and UCSF classifications and would improve the accuracy of radiological staging while preserving equivalent and acceptable rates of tumor recurrence and post-transplant patient survival.

Acknowledgement

The authors would like to thank Abdul Salam, MSc for the statistical support.
References


3. Patient selection based on Total Tumor Volume and Alpha Fetoprotein, a registry-based study

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by Christian Toso, Sonal Asthana, David L. Bigam, A.M. James Shapiro, Norman M. Kneteman
Abstract

The current model of liver graft allocation in place in the United States favors transplantation of patients with small hepatocellular carcinomas (HCCs) within the Milan criteria (a single tumor up to 5 cm in diameter or up to three lesions, none larger than 3 cm). Although several reports have suggested that these criteria could be extended, there is currently no agreement on new selection tools. In this study, we performed an overview of 6478 adult recipients of an isolated first liver transplant registered in the Scientific Registry of Transplant Recipients (SRTR) database. From March 2002 to January 2008, increasing numbers of patients outside Milan criteria (P ≤0.001) have been registered for a transplant, but they still represent less than 5% of the transplants performed for HCC. Of all the tested variables (tumor number, largest tumor size, and Milan and University of California San Francisco criteria), only total tumor volume (TTV; P ≤0.05) and alpha fetoprotein (AFP; P ≤0.001) could predict patient survival. While these two parameters demonstrated independent behaviors (no patient demonstrated an increase in both values), a composite score was defined, with patients with a TTV >115 cm$^3$ or an AFP >400 ng/mL being outside criteria. The combined TTV/AFP score efficiently predicted posttransplant survival (hazard ratio = 2, 95% confidence interval = 1.7-2.4, P ≤0.001); patients not meeting these criteria had a survival below 50% at 3 years. Conclusion: According to the present SRTR data, Milan criteria are too restrictive, and patients with larger TTV can enjoy satisfactory posttransplant survivals. A composite patient selection
score combining TTV and AFP was the most effective of all tested staging criteria for the prediction of posttransplant patient survival for candidates with HCC.
Introduction

Since the introduction of the Milan criteria in 1996, liver transplantation has progressively developed recognition as the best treatment option for patients with small unresectable hepatocellular carcinomas (HCC)(17). These criteria allow transplantation for patients with a single HCC up to 5 cm in diameter or up to 3 HCCs none larger than 3 cm. Results in patient cohorts within the Milan criteria have now been reproduced by several centers (39-44) and these criteria have been adopted by the United Network for Organ Sharing (UNOS) for allocation of organs in the United States.

While excellent results can be achieved when respecting Milan criteria, many recent data have suggested that they are too restrictive and that similar “acceptable” outcomes can be achieved with more liberal selection policies (45). The first well structured evidence was reported by Yao et al from the University of California, San Francisco (UCSF), demonstrating maintainence of good outcomes with transplantation of patients with a single HCC up to 6.5 cm in diameter or up to 3 HCCs, none larger than 4.5 cm and with a cumulative diameter up to 8 cm (40). While their first study was based on explant tumor characteristics, the same group demonstrated more recently that similar excellent outcomes (93.6% of patients free of recurrence at 5 years) can be achieved based on prospective radiological data (46).

Despite validation by several other groups (41, 47), the UCSF criteria have not been unanimously accepted yet. This may be related to the fact that a large multicenter study suggested decreased outcomes in patients between Milan and
UCSF criteria (48). In addition, UCSF criteria allow transplantation for large HCCs (6.5 cm, representing a tumor volume of 144 cm³), which have been associated with decreased outcomes in several studies (49-51). UCSF criteria (like Milan) also exclude all patients with more than three lesions, some of whom may have the potential to enjoy good outcomes if tumors remain of reasonable size (42, 43, 45, 52).

We have recently demonstrated in a three-center study including 288 patients that the total tumor volume (TTV) was the most accurate morphological criteria to select HCC patients prior to transplant. Those with a TTV up to 115 cm³ demonstrated 79% 5-year survival, without tumor number restriction (52).

The present large registry-based study intended to perform an overview of the liver transplants reported in the United States and to assess morphological (tumor size, number, TTV) and biological (alpha feto-protein, AFP) criteria which could help selection of the most appropriate transplant recipients from the population with HCC.
Patients and Methods

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN), and has been described elsewhere (53). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors. The study has been reviewed and approved by the Health Research Ethics Board at the University of Alberta. The study population included all adult (≥18 years) patients, who received a first isolated liver transplant from March 2002 to January 2008 for a diagnosis of HCC.

The following variables were used to predict patient survival: number of HCC, size of the largest tumor, Milan and UCSF criteria, TTV and AFP. Data obtained on the date closest to transplant were used. The mean time between AFP measurement and transplant was 3 ±2.7 months. Number and size variables were based on pre-transplant radiological assessment and were available in all patients. The type of diagnosis modality used was reported in 5220 patients (80.5%). They included computed tomography (58.7%), magnetic resonance imaging (42.5%), biopsy (10.6%), ultrasound (8.3%) and /or angiogram (4.7%) performed 3.6 ±3.7 months prior to transplant.

Milan criteria included patients with a single tumor up to 5 cm in diameter or up to 3 tumors none larger than 3 cm (17). UCSF criteria included patients with a
single tumor up to 6.5 cm diameter or up to 3 tumors, none larger than 4.5 cm and with a cumulative diameter up to 8 cm (40). TTV was calculated as the sum of the volume of each tumor \((\frac{4}{3})\pi r^3\) based on the maximum radius of each tumor (52).

The occurrence and the date of death were obtained from data reported to the SRTR by the transplanting centers and were completed by data from the US Social Security Administration and from the OPTN. All deaths were taken into account in the analysis, including those associated to HCC or not. Data related to HCC recurrence was not consistent (low granularity and not previously validated) and have not been included in the analysis. As a consequence, some patients may have been alive with an HCC recurrence and were not considered as an event in the survival analyses.

A preliminary univariate analysis was performed using the Kaplan-Meier method and comparing groups using the log-rank test. Cox regression was used to estimate covariate adjusted hazard ratios (HR) of variables with a significance of at least 0.05 on the univariate analysis. Covariates included MELD, date of transplant, age at transplant, gender, race, primary underlying liver disease and pre-transplant tumor treatment (yes vs. no). Statistical processing was performed in patients with a complete set of variables for each specific analysis.

Further analyses used a Chi-square test to assess the ratio of patients transplanted outside Milan criteria according to the year of transplant. Results were provided as mean ± standard deviation. Standard alpha level of 0.05
indicated statistical significance. Analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL).
Results

During the study period, 6478 adult patients received an isolated first liver transplantation and were registered in the SRTR database. Their characteristics were reported in Table 3.1. The population included mainly men (77%) and had a mean age of 56 ±8 years. The most frequent underlying liver disease was related to hepatitis C virus infection (49.5%). Median follow-up (till death or last data report) was 13.4 [0-67.9] months.
<table>
<thead>
<tr>
<th>Patients (number)</th>
<th>6478</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>56 ±8</td>
</tr>
<tr>
<td>Gender</td>
<td>female:1477/ male:5001</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>4325 (67)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>899 (14)</td>
</tr>
<tr>
<td>Asian</td>
<td>662 (10)</td>
</tr>
<tr>
<td>African American</td>
<td>531 (8)</td>
</tr>
<tr>
<td>Other/Multi-race</td>
<td>61 (1)</td>
</tr>
<tr>
<td>Cause of liver disease (%)</td>
<td></td>
</tr>
<tr>
<td>HCV (±alcohol, ±HBV)</td>
<td>3221 (49.5)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>538 (8)</td>
</tr>
<tr>
<td>HBV</td>
<td>392 (6)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>249 (4)</td>
</tr>
<tr>
<td>NASH</td>
<td>78 (1)</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>73 (1)</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>70(1)</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>57 (1)</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>49(1)</td>
</tr>
<tr>
<td>Alpha1-antitrypsin deficiency</td>
<td>25(0.5)</td>
</tr>
<tr>
<td>Metabolic disorder</td>
<td>15 (0.5)</td>
</tr>
<tr>
<td>Acute liver necrosis</td>
<td>228 (3.5)</td>
</tr>
<tr>
<td>Other</td>
<td>1483 (23)</td>
</tr>
<tr>
<td>Pre-transplant treatment (%)</td>
<td>2163 (33)</td>
</tr>
<tr>
<td>Outside Milan criteria (%)</td>
<td>205 (3.2)</td>
</tr>
<tr>
<td>Outside UCSF criteria (%)</td>
<td>51 (0.8)</td>
</tr>
<tr>
<td>Total Tumor Volume &gt;115 cm³ (%)</td>
<td>18 (0.3)</td>
</tr>
<tr>
<td>Mean serum alpha fetoprotein level (ng/ml)</td>
<td>262 ±1473</td>
</tr>
<tr>
<td>Serum alpha fetoprotein level &gt;400 (%)</td>
<td>465 (8.9)</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus infection, HBV: hepatitis B virus infection

Table 3.1: Patient and tumor characteristics
Complete study variables were available for 80.4% of the patients. The most frequently missing variable was AFP, not reported in 1260 patients (19.5%). The mean diameter of tumors was 2.3 ±1.1 cm (largest diameter: 8 cm). The mean number of tumors was 1.4 ±0.7 (largest number: 9 tumors). Most of the patients were reported to fulfill Milan criteria (96.8%). However, an increasing number of patients outside Milan criteria were transplanted over time (p<0.001, Figure 3.1A). As expected, the TTV followed the same trend and more patients with high cumulative tumor volumes were transplanted over time from 2003 to 2007 (Figure 3.1B).
Figure 3.1: Rate of patients transplanted outside Milan criteria and registered in the SRTR database from 2003 to 2007; \( p \leq 0.001 \) (A). Evolution of the Total Tumor Volume of liver recipients registered in the SRTR database from March 2002 to January 2008.
Number of tumors, size of the largest tumor as well as Milan and UCSF criteria were not predictive of survival in the univariate analysis (Table 3.2). TTV was studied with a cut-off of 115 cm$^3$, which was established in a previous study (52). This value was based on the risk of recurrence in our own recipient population at the University of Alberta and was obtained with use of a receiver operating characteristic (ROC) curve (area under the curve: 0.8). This cut-off was validated in a multi-center study, assessing the risk of recurrence and survival (52). In the present SRTR data, TTV again was predictive of patient survival (p≤0.05, Table 3.2) as also was AFP (p≤0.001).
<table>
<thead>
<tr>
<th></th>
<th>Three-year patient survival (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70.9</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>72.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>73.2</td>
<td></td>
</tr>
<tr>
<td>&gt;3</td>
<td>56.6</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>71.6</td>
<td>0.59</td>
</tr>
<tr>
<td>&gt;5</td>
<td>67.4</td>
<td></td>
</tr>
<tr>
<td>Milan criteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulfilled</td>
<td>71.7</td>
<td>0.25</td>
</tr>
<tr>
<td>Beyond</td>
<td>64.9</td>
<td></td>
</tr>
<tr>
<td>UCSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulfilled</td>
<td>71.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Beyond</td>
<td>61.8</td>
<td></td>
</tr>
<tr>
<td>Total Tumor Volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤115</td>
<td>71.6</td>
<td>≤0.05</td>
</tr>
<tr>
<td>&gt;115</td>
<td>41.7</td>
<td></td>
</tr>
<tr>
<td>Alpha feto-protein level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>71.6</td>
<td>≤0.001</td>
</tr>
<tr>
<td>&gt;10, ≤100</td>
<td>71.3</td>
<td></td>
</tr>
<tr>
<td>&gt;100, ≤500</td>
<td>59.2</td>
<td></td>
</tr>
<tr>
<td>&gt;500</td>
<td>50.7</td>
<td></td>
</tr>
</tbody>
</table>

Milan criteria as previously defined by Mazzaferro et al (1)
UCSF criteria as previously defined by Yao et al. (3)
Total Tumor Volume as previously defined by Toso et al. (15)

Table 3.2: Univariate analysis of factors impacting on patient survival
The survival of patients within Milan criteria was similar to that of patients beyond Milan, but within UCSF ($p=0.6$) and to those beyond UCSF, but with a $TTV \leq 115$ cm$^3$ ($p=0.6$, Figure 3.2). Only patients with a $TTV > 115$ cm$^3$ demonstrated a significantly lower survival ($p \leq 0.05$).

Figure 3.2: Survival after liver transplantation for patients within Milan criteria; outside Milan but within UCSF; outside UCSF but with a $TTV \leq 115$ cm$^3$; or with a $TTV > 115$ cm$^3$. All survivals were similar to the one of patients within Milan, except for patients with $TTV > 115$ cm$^3$ ($p \leq 0.05$).
When performing a multivariate analysis, corrected for MELD, date of transplant, age at transplant, gender, race, primary liver disease and the use of a pre-transplant tumor treatment, both TTV (HR=1.4 for TTV/100, p≤0.05) and AFP (HR=1.1 for AFP/1000, p≤0.001) remained significant in predicting patient survival (Table 3.3).

After plotting TTV and AFP values, we could identify three groups of patients, namely those with both low TTV and low AFP, and those with either a high TTV or a high AFP (Figure 3.3A). None of the reported patients had both a high TTV and a high AFP. We therefore performed the subsequent analysis using a combined TTV/AFP score, defining patients with a TTV >115 cm$^3$ or an AFP >400 ng/ml as outside criteria. The TTV cut-off was reported from our previous report and corresponds to a single tumor of approximately 6 cm in maximum diameter or three lesions of approximately 4.2 cm (52). The AFP cut-off of 400 ng/ml was selected from several published data (44, 54, 55), as well as from our own series (ROC curve area under the curve of 0.7 when looking at recurrence) (52). Among patients within Milan, 8.8% were over the AFP cut-off. The TTV/AFP score was most accurate to predict patient survival (HR=2, 95% CI=1.7-2.4, p≤0.001). Patients with a high TTV or a high AFP had a corrected three-year survival lower than 50% (Figure 3.3B). Four-hundred seventy nine recipients (7.4%) were included in this group.
Figure 3.3: Distribution of TTV and AFP for each patient transplanted with a liver for HCC and registered in the SRTR database from March 2002 to January 2008 (A). Corrected patient survival for recipients with TTV≤115cm$^3$ and AFP≤400ng/ml vs. TTV>115cm$^3$ or AFP>400ng/ml; p≤0.001 (B). Survival was corrected for MELD, date of transplant, age at transplant, gender, race, primary underlying liver disease and pre-transplant tumor treatment (yes vs. no).
Discussion

According to the present SRTR data, Milan criteria are too restrictive and patients with larger TTV can enjoy satisfactory post-transplant survivals. A combined patient selection score based on TTV and AFP was the most predictive of all tested criteria sets when applied to the SRTR data.

Over 95% of the liver transplantations performed for HCC and registered in the SRTR database were done for patients fulfilling Milan criteria. We observed however a significant increase over time in the rate of recipients with large TTV, exceeding Milan criteria. This observation may reflect a true increase in the number of such transplants and/or a more accurate reporting of them by the centers. Both explanations can be viewed as an overall agreement that Milan criteria are too restrictive and that liver transplants can be performed safely in selected patients with larger and/or more numerous HCC. This point of view is shared by many reports (40-43, 45-47, 52). It is also supported by the present data, as patients if TTV up to 115 cm$^3$ demonstrated similar survival as those respecting Milan or UCSF criteria (Figure 2).

In the present registry-based study, both Milan and UCSF criteria failed to predict patient survival. This observation needs to be taken with caution, as tumor size and number were entered by the centers in order to obtain exception MELD points, which may introduce reporting bias. This said, while Milan, UCSF and TTV were all based on the same reported numbers, only TTV remained powerful in selecting patients for transplant. This suggests the superiority of this score and
is in support of our previously reported observations (52). Of note, this was achieved despite the proportionally low number of patients transplanted with large TTV.

The main advantage of TTV is that it does not include a rigid tumor number limit, unlike Milan and UCSF criteria. Such a limit prevents transplantation of patients with more than three lesions, even if they are of very small size and would be associated with good post-transplant outcome (42, 43, 45, 52). In addition, the accuracy of the pre-transplant radiological assessment is substantially improved by using TTV, as compared to Milan or UCSF, thus achieving better patient selection with pre-transplant imaging studies (52). This is related to the fact that TTV gives more power to larger tumors as the calculated volume increases much faster than the related diameter \((4/3)\pi r^3\), and the sensitivity and specificity of radiology is higher for larger tumors (11).

AFP was the other variable highly predictive of patient survival. This observation confirms several previous studies (44, 49, 56, 57). An AFP value was available for more than 80% of the studied transplants, reflecting the good granularity of the database (80.4% of cases had complete study variable). In addition, as AFP does not enter into patients selection for transplantation at the present time, the registered data were likely very accurate.

Because of the lack of accurate data on HCC recurrence or on the presence of HCC at the time of death, we could not determine cut-off values for TTV or AFP from the present registry. We therefore used cut-offs at 115 cm\(^3\) for TTV and 400 ng/ml for AFP, which were determined from our own patient population using
ROC curves based on the risk of HCC recurrence (52). The areas under the curve were 0.8 for TTV and 0.7 for AFP (52). The same AFP cut-off has also been selected by several other groups (44, 54, 55).

A combination of TTV and AFP appears logical when looking at their distribution on Figure 3. There is no linear correlation between them in the present population of transplant recipients, suggesting that they behave independently. The absence of reported cases with both a high tumor volume and a high AFP, may be linked to the fact that these patients have particularly aggressive tumors, which are rapidly progressive, preventing listing and /or transplantation. While TTV provides a morphological limit to HCC selection, AFP brings a simple assessment of tumor biology. The combined score would thus exclude patients with large HCC or with small HCC with a potentially aggressive behavior, who have a corrected patient survival lower than 50% at 3 years.

Several potential variables were not assessed by the present study. Macrovascular tumor invasion has been recognized by several previous study as being associated with poor post-transplant outcome (49, 52). The TTV/AFP selection score should therefore only be applied to patients without sign of vascular invasion by HCC on radiology. Biopsy-based factors, such as tumor grade would also be of interest (40, 44, 52, 58), but they have several negative points, which challenge their applicability. An HCC biopsy bears a risk of tumor seeding. In addition, large HCCs are not homogeneous and the risk of needle biopsy sampling error is real. Finally, these risks would be multiplied in case of
multiple lesions, rendering the practicability of the biopsy difficult (patients with up to nine lesions were included in the present study).

It is difficult to estimate how the TTV/AFP score would impact on the number of patients recruited for transplantation, because the studied registry population was highly pre-selected. As demonstrated in a previous study, TTV (cut-off 115 cm$^3$) alone would increase the number of candidates by 28-53% compared to Milan and 16-26% compared to UCSF (52). On the other side, AFP (cut-off 400 ng/ml) would have excluded 8.8% of the patients meeting Milan criteria in the present study. We can therefore speculate that the combined score would lead to a marginal net increase in the number of candidates.

Such an increase in the requirement of liver grafts would certainly represent a major challenge considering the limited organ resources (59). On the other hand, not offering a transplant to patients who have the potential to have good outcomes is ethically disturbing.

Taken together, the presented registry-based data suggest that Milan criteria are too restrictive and that a combined patient selection score based on TTV and AFP would be more appropriate for patient selection of liver transplantation in the presence of HCC.
Acknowledgements

Special note: The data reported here have been supplied by the Arbor Research Collaborative for Health (Arbor Research) as the contractor for the Scientific Registry of Transplant Recipients (SRTR). The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.
References


4. The estimated number of patients selected for transplantation using expanded selection criteria

A version of this chapter has been published in Transplant International 2009; 22(9): 869-75

by Christian Toso, Norman M. Kneteman, A.M. James Shapiro, David L. Bigam
Abstract

Recently, several groups have introduced expanded criteria for selection of patients with hepatocellular carcinoma (HCC) prior to transplant, but the exact number of potential newly recruited patients remains unclear. This registry-based study assessed 270 patients diagnosed with HCC. The potential number of transplant candidates was based on age ≤65 years), absence of metastases and macro-vascular invasion, and on 12 previously published, expanded selection criteria. A wide range of increase in the number of transplant candidates was observed (12-63% when compared with the number of such candidates who would have been selected solely based on the Milan criteria). The most conservative criteria were Seoul (Kwon, 2007; increase of 12%), Valencia (Silva, 2008; 16%), total tumor volume/alpha-fetoprotein (Toso, 2009; 20%) and UCSF (Yao, 2007; 20%). This data will assist Centers and policy agencies in predicting the need for resources linked to an expansion of criteria.
Introduction

Since the publication of the Milan criteria in 1996, transplantation has been recognized as the best treatment for patients with small non-resectable HCC’s (17). When respecting these criteria, excellent outcomes can be achieved with five-year survival rates between 70 and 90%, and most centers have adopted this score to select transplant candidates (17, 18, 40, 42-44, 46, 54, 60-68). Several studies however have now demonstrated that the Milan criteria are too restrictive and that favorable outcomes can be achieved following more liberal selection policies (18, 45). The group at the University of California, San Francisco (UCSF), was the first to propose expanded criteria (40). While validated by other centers (41, 46, 48, 56), the UCSF criteria have failed to gain unanimous recognition, possibly because they exclude patients with more than three HCC’s (even when of small size and with expected favorable outcomes) and include patients with large tumors (up to 6.5 cm in diameter), which have been associated with decreased post-transplant outcomes (49-51). In addition, a large multicenter study has suggested decreased outcomes in patients between Milan and UCSF criteria (48). As a consequence, many other groups have proposed alternative morphological scores to select transplant candidates (Table 1)(18, 42-44, 54, 60-66, 69). The backbone of most of these scores is a combination of HCC size and number, with or without the addition of alpha-fetoprotein (AFP) or tumor grade. We recently introduced the concept of total tumor volume (TTV ≤115 cm³) for patient selection, and demonstrated similar
outcomes to Milan and UCSF criteria, but with the inclusion of more patients (66, 70).

The acceptance of new expanded selection criteria should be based on the observation that the newly recruited patients (beyond Milan, but within the new score) have stable and acceptable post-transplant outcomes compared to those within Milan criteria. Beside this, it is important for Centers and policy agencies to be able to predict the need for resources linked to an expansion of criteria, as this has the potential to impact the work load as well as the need for alternative sources of organs, including live donors or donors after cardiac death. The present study examined the estimated impact of various previously proposed expanded criteria on the number of newly recruited transplant candidates.
Methods

This study was based on data from the Alberta Cancer Registry (ACR), which is a population-based registry, which records and maintains data on all new cancer cases and deaths occurring within the province of Alberta, Canada. It is operated by the Alberta Cancer Board’s Division of Population Health and Information and is mandated by the Cancer Programs Act of Alberta. It is mandatory for all physicians in Alberta to contribute all cancer patients to the database. The ACR has met the Gold Standard for Registry Certification for the years included in the study, representing the highest North American Association for Central Cancer Registries standard for complete, accurate, and timely data (including completeness of data of 95% or higher, www.naaccr.org). This study has been reviewed and approved by the Health Research Ethics Board at the University of Alberta.

All patients, diagnosed with HCC between January 2003 and December 2006 in Northern Alberta (Edmonton and area, 1.6 million inhabitants in 2006) were included. Data from the ACR included basic demographic (identifier, gender, date of birth, date of death) and cancer-related information (date of HCC diagnosis, modality of diagnosis including radiology, histology or post-mortem, and histological grade when available). The registry database was completed by a chart review assembling data on the cause of underlying liver disease, size and number of HCCs, AFP level and administration of a local HCC treatment, including surgical resection, trans-arterial chemoembolization (TACE), ethanol injection or radiofrequency ablation (RFA). Charts were available on all patients.
The morphological staging was assessed from reports of ultrasound, contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI). A lesion was considered an HCC, when it demonstrated an arterial enhancement with wash-out on delayed images, or when significant increase in size was documented. A stable, non-enhancing small lesion was not considered as HCC. Morphological and AFP data were recorded from the time of diagnosis to the end of follow-up (defined as death or September 2008). A successful down-staging after local HCC treatment was defined as the absence of enhancement around the site of ablation for at least three months, as described by Yao et al (18, 71). Of note, the policy in place in the region was to offer local HCC treatment any time tumor characteristics and general patient condition allowed.

Potential candidates for transplantation were defined as ≤65 years of age and without metastases or large liver vessel tumor invasion. Following this first candidate selection, further assessments were performed according to previously proposed selection criteria summarized in Table 4.1.
<table>
<thead>
<tr>
<th>Original author (year), abbreviation</th>
<th>Criteria</th>
<th>Reported 5-year patient survival</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mazzaferro (1996), Milan</td>
<td>1 HCC ≤5 cm or ≤3 HCC ≤3 cm</td>
<td>85%&lt;sup&gt;1&lt;/sup&gt; (35)</td>
<td>(1)</td>
</tr>
<tr>
<td>Yao (2001), UCSF</td>
<td>1 HCC ≤6.5 cm or ≤3 HCC ≤4.5 cm with cumulated diameter ≤8 cm</td>
<td>Milan: 72% (46)</td>
<td>(2)</td>
</tr>
<tr>
<td>Herrero (2001), CUN</td>
<td>1 HCC ≤6 cm or ≤3 HCC ≤5 cm</td>
<td>Milan: 70% (59)</td>
<td>(3,10)</td>
</tr>
<tr>
<td>Yao (2007), UCSF</td>
<td>Same as Yao (2001)</td>
<td>Milan: 80%&lt;sup&gt;2&lt;/sup&gt; (130)</td>
<td>(4)</td>
</tr>
<tr>
<td>Onaca (2007), Dallas</td>
<td>1 HCC ≤6 cm or ≤4 HCC ≤5 cm</td>
<td>Milan: 62%&lt;sup&gt;2&lt;/sup&gt; (628)</td>
<td>(5)</td>
</tr>
<tr>
<td>Kwon (2007), Seoul</td>
<td>HCC ≤5 cm without # restriction and AFP ≤400 ng/ml</td>
<td>Milan: 80% (99)</td>
<td>(6)</td>
</tr>
<tr>
<td>Sugawara (2007), Tokyo</td>
<td>≤5 HCC ≤5 cm</td>
<td>Milan: 94%&lt;sup&gt;3&lt;/sup&gt; (68)</td>
<td>(7)</td>
</tr>
<tr>
<td>Takada (2007), Ito</td>
<td>≤10 HCC ≤5 cm</td>
<td>Milan: 73% (74)</td>
<td>(8,9)</td>
</tr>
<tr>
<td>Zheng (2008), Hangzhou</td>
<td>Total tumor diameter ≤8 cm or HCC grade I or II and AFP ≤400 ng/ml</td>
<td>Milan: 76% (152)</td>
<td>(12)</td>
</tr>
<tr>
<td>Lee (2008), Asan</td>
<td>≤6 HCC ≤5 cm</td>
<td>Milan: 78.3% (72)</td>
<td>(13)</td>
</tr>
<tr>
<td>Silva (2008), Valencia</td>
<td>≤3 HCC ≤5 cm with cumulated diameter ≤10 cm</td>
<td>Milan: 62% (231)</td>
<td>(14)</td>
</tr>
<tr>
<td>Toso (2008), TTV</td>
<td>TTV ≤115 cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Milan: 78% (157)</td>
<td>(15)</td>
</tr>
<tr>
<td>Mazzaferro (2009), Up-to-seven</td>
<td>Number + maximum size of HCC = 7</td>
<td>Milan: 73% (444)</td>
<td>(16)</td>
</tr>
<tr>
<td>Toso (2009), TTV/ AFP</td>
<td>TTV ≤115 cm&lt;sup&gt;3&lt;/sup&gt; and AFP ≤400 ng/ml</td>
<td>Milan: 71%&lt;sup&gt;3&lt;/sup&gt; (6268)</td>
<td>(17)</td>
</tr>
</tbody>
</table>

<sup>1</sup> four-year survival; <sup>2</sup> recurrence-free survival; <sup>3</sup> three-year survival

EC: beyond Milan and within expanded criteria, TTV: total tumor volume

AFP: alpha fetoprotein, NA: not available

**Table 4.1: Selected transplant criteria**
We first included all potential candidates in the analysis. In a subsequent model, we excluded patients with preserved hepatocellular function, who have undergone HCC resection, assuming that they would be better treated by this modality (resection was the first choice when feasible). The impact of down-staging was also further estimated.

Results were provided as mean ± standard deviation. Categorical tables and Chi-square tests were used to compare criteria. Standard alpha level of 0.05 indicated statistical significance. Analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL).
Results

During the four-year study period, 270 patients were diagnosed with HCC in Northern Alberta, representing an incidence of 4.2/100,000 inhabitant-year. Of them, 129 (48%) were >65 years old and were not considered potential transplant candidates (Figure 4.1). Of the remaining 141 patients, 14 (10%) had radiological evidence of major vascular invasion, six (4%) had metastases and two (1.5%) had both macro-vascular invasion and metastases. In addition, 11 patients had incomplete data, due to either very advanced tumors and/or death directly after diagnosis, and therefore were not included in the analysis. Of note, they would not have been transplant candidates.
270 patients with HCC

Non-transplant candidates:
129 (48%) age >65 years

22 (15.5%) with metastasis or tumoral thrombosis of a large hepatic vessel

11 patients with incomplete assessments

108 patients potentially available for transplantation

Figure 4.1: Selection of potential transplant candidates with HCC.
The remaining 108 patients with HCC, with complete data, were further considered for transplantation (Table 4.2). This group was comprised of 81% males, with a mean age of 55 ±5 years. The most frequent cause of underlying liver disease was related to hepatitis C virus infection (57%). The mean number of HCC was 2 ±2 (range 1-9), mean diameter of the largest HCC 5 ±4 cm (0.6-21) and mean TTV 294 ±904 cm³ (0.11-7239). AFP demonstrated a wide range from one to 4500 ng/ml (mean 542 ±1067 ng/ml).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (number)</td>
<td>108</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>55 ±5</td>
</tr>
<tr>
<td>Gender</td>
<td>female:20/ male:88</td>
</tr>
<tr>
<td>Cause of liver disease (%)</td>
<td></td>
</tr>
<tr>
<td>HCV (±alcohol, ±HBV)</td>
<td>62 (57)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>12 (11)</td>
</tr>
<tr>
<td>HBV</td>
<td>14 (13)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>8 (7.5)</td>
</tr>
<tr>
<td>NASH</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (7.5)</td>
</tr>
<tr>
<td>Local HCC treatment (%)</td>
<td></td>
</tr>
<tr>
<td>Surgical resection</td>
<td>19 (18)</td>
</tr>
<tr>
<td>TACE</td>
<td>17 (16)</td>
</tr>
<tr>
<td>Ethanol injection</td>
<td>11 (10)</td>
</tr>
<tr>
<td>Radiofrequency ablation</td>
<td>8 (7.5)</td>
</tr>
<tr>
<td>None</td>
<td>60 (56)</td>
</tr>
<tr>
<td>Mean number of tumors (#)</td>
<td>2 ±2</td>
</tr>
<tr>
<td>Mean diameter of the largest tumors (cm)</td>
<td>5 ±4</td>
</tr>
<tr>
<td>Mean total diameter (cm)</td>
<td>7 ±6</td>
</tr>
<tr>
<td>Mean Total Tumor Volume (cm³)</td>
<td>294 ±904</td>
</tr>
<tr>
<td>Mean serum alpha fetoprotein level (ng/ml)</td>
<td>542 ±1067</td>
</tr>
<tr>
<td>Serum alpha fetoprotein level &gt;400 (%)</td>
<td>23 (21)</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus infection, HBV: hepatitis B virus infection
TACE: transarterial chemo-embolization

Table 4.2: Characteristics of potential transplant candidates
Of the remaining 108 patients with HCC, 49 (45%) fulfilled Milan criteria. These potential transplant candidates within this criteria represented 18% of all patients diagnosed with HCC during the study period (49/270). By applying the range of proposed expanded selection criteria, the number of potential transplant candidates increased by 12-63% compared to Milan (Figure 4.2A). The most conservative selections were performed with Seoul (increase of 12% compared to Milan), Valencia (16%), TTV/AFP (20%) and UCSF (20%) criteria. In a subsequent analysis, patients who had undergone surgical resection of HCC were presumed to have been cured and were no longer considered for transplant (Figure 4.2B). Once again, a wide range of increase was observed compared to the Milan criteria (17.5-62.5%) and the most conservative were the Seoul (17.5%), Valencia (20%), TTV/AFP (25%) and UCSF (17.5%) criteria. Forty-eight patients (44%) underwent local HCC treatment. The impact of the expanded selection criteria was assessed allowing down-staging to any criteria (Figure 4.2C). Seven patients were successfully down-staged to within the Milan criteria and remained stable for more than three months, increasing the number of potential candidates within the Milan criteria to 56. After down-staging, the number of potential transplant candidates was expanded by 5-38% compared to Milan. Once again, the most conservative were Seoul (5%), Valencia (7%), TTV/AFP (9%) and UCSF (5%) criteria.
Increase in number of HCC transplant candidates compared to Milan, allowing for down-staging (%)

Increase in number of HCC transplant candidates compared to Milan, not including patient with HCC resection (%)

Increase in number of HCC transplant candidates compared to Milan (%)

References:
- Mazzaferro (1996), Milan
- Kwon (2007), Seoul
- Silva (2008), Valencia
- Toso (2009), TTV/AFP
- Yao (2001), UCSF
- Herrero (2001), CUN
- Sugawara (2007), Tokyo
- Lee (2008), Asan
- Takada (2007), Ito (2007), Kyoto
- Mazzaferro (2009), Up-to-seven
- Onaca (2007), Dallas
- Toso (2008), TTV
- Zheng (2008), Hangzhou
Figure 4.2: Estimated increase in the number of transplant candidates with HCC as compared to the number of patients within the Milan criteria. The analysis described in (A) was performed using all patients with HCC, ≤65 year-old and without evidence of macro-vascular invasion or metastases. The analysis in (B) excluded patients with preserved hepatocellular function, who had undergone HCC resection, assuming that they had attained cure by this modality. The estimated described in (C) allowed down-staging, defined as the absence of enhancement around the site of ablation for at least three months.
In order to better understand the observed wide range of expansion in the number of potential transplant candidates, we plotted the allowed number of tumors, diameter and TTV for each criterion (Figure 4.3). While the allowed maximum HCC diameter was consistently between 5 and 6 cm (except Hangzhou with 8 cm), a wide range of tumors number was allowed (from 3 to no restriction).
Figure 4.3: Allowed maximum number of HCC and HCC diameter for the various studied criteria. The criteria were put in the same order as in Figures 1. Arrows reflect the absence of a set upper limit.
Discussion

This registry-based study estimates that the number of HCC transplant candidates would increase by 12-20% with the application of conservative (Seoul, Valencia, TTV/AFP and UCSF) and by up to 63% with less conservative (Hangzhou) selection criteria. Until now, the expected number of newly recruited HCC transplant candidates could not be accurately predicted as most previous studies were retrospective or based on highly pre-selected patient populations. Conversely, the present work provides an estimate of the potential HCC transplant candidate pool, utilizing all patients diagnosed with HCC within a defined region. Of note, the presently reported incidence of HCC (4.2/100,000) matches previous reports from similar populations, including all of Canada, the United Kingdom and the United States of America, with incidences <5/100,000 (72), which should be viewed as a validation of the quality of the Alberta Cancer Registry database. While several factors could be accurately quantified (patient age, presence or absence of macro-vascular tumor invasion or of metastases), others like alcohol abstinence or the presence of medical co-morbidities were not taken into account. This is related to the fact that most patients included in the present study, did not undergo a formal transplant assessment, and that these potential contra-indications could not be accurately identified. Both the alcohol abstinence and medical co-morbidities however are independent from the number and the size of HCCs. We can therefore be confident that patients with such contra-indications were distributed homogeneously within the studied population and
that the reported rates of increase in the number of HCC transplant candidates were accurate.

The 12 expanded selection criteria that were studied lead to an extremely wide variety of increase in the number of transplant candidates with HCC. The most conservative ones, Seoul, Valencia, TTV/AFP and UCSF, induced an increase of 12-20% as compared to Milan (40, 46, 54, 65, 73). Conversely, Dallas, TTV and Hangzhou criteria lead to 41-63% increase (44, 61, 66). We believe that the selection of new expanded selection criteria should be based solely on the observation that the newly recruited patients (beyond Milan, but within the new score) have stable and acceptable post-transplant outcomes as compared to those within Milan criteria. As such, the data included in the present study should not be viewed as arguments to favor one score or the other. They will however help Centers and policy agencies in predicting potential increase in the number of transplants and the associated work load. Both will require appropriate planning, including potentially looking for alternative sources of organs such as live donors or donors after cardiac death.

**Acknowledgements**

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References


5. The place of downstaging for hepatocellular carcinoma

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Abstract

In the treatment of hepatocellular carcinomas, therapies such as trans-arterial chemo-embolisation, trans-arterial radioembolisation, percutaneous ethanol injection and radio-frequency ablation can decrease the size (and overall viability) of the tumours, thus potentially increasing the proportion of patients qualifying for resection and transplantation. While the use of such downstaging therapies is straightforward when resection is the aim, in a similar way to other neo-adjuvant treatments in the surgery of tumours that are too large or awkwardly placed to be primarily resected the issues related to transplantation are more complex. In the context of transplantation the word "downstaging" designates not only a neo-adjuvant treatment, but also a selection strategy to allow patients who are initially outside accepted listing criteria to benefit from transplantation should the neo-adjuvant therapy be successful in reducing tumour burden. The effectiveness of downstaging as a selection strategy, at first questioned because of methodological bias in the studies that described it, has been recently demonstrated by more solid prospective investigations. Several issues however remain open, such as inclusion criteria before the strategy is implemented (size/number, surrogate markers of differentiation/vascular invasion such as alpha-fetoprotein), the choice of which downstaging therapy, the endpoints of treatment, and the need and duration of a period of observation proving disease response or stabilisation before the patient can be listed. The present review discusses which treatments and strategies are available for downstaging HCC on the basis of the published literature.
**Introduction**

Curative surgical treatments for patients with hepatocellular carcinoma (HCC) include resection and transplantation. Resection can be performed in patients with good liver function and localised HCCs, while transplantation is favoured in selected patients with decreased liver function and/or multiple nodules. Over the years, the place of these therapies has been well defined, but they can only be attempted in 10-20% of patients with HCC, as in the majority, the disease will be too advanced (5, 74, 75)]. A broader use of local HCC treatments has the potential to shrink the tumour and allow a curative option in patients for whom tumour size or location next to vital anatomical structures is the limiting factor. These treatments include transarterial chemo-embolisation (TACE), radio-frequency ablation (RFA), percutaneous ethanol injection (PEI) and transarterial radioembolization (TARE).

The present review article discusses the use of such local HCC treatment prior to surgery or transplantation, and the place that these treatments have taken in transplant candidates as a selection tool that refines the usual criteria based on number and size.
Neoadjuvant treatment vs. downstaging: a more strict definition

The word *downstaging* is used loosely to qualify any type of treatment aiming to control tumour growth prior to surgery, with a confusing overlap with the term *neo-adjuvant treatment*. In this review we suggest restricting the use of the word *downstaging* to the aim or the result of a treatment that intends to facilitate or make possible a surgical procedure that would otherwise be too risky or unfeasible. *Neoadjuvant treatment* can be given to patients in whom the procedure can be done primarily, with aims that may be different from downstaging, such as to improve the long-term results, or to limit the complications during the time waiting for the procedure to be done. While *neo-adjuvant* treatments often refer the use of systemic drugs, aiming at controlling both the primary lesion and circulating cancer cell, it will here be applied to local HCC therapies.

The aims of neoadjuvant treatments and of downstaging are different in patients who are candidate for resection or for transplantation (Figure 5.1). Before resection, *neo-adjuvant treatment* can be given with the aim to improve the results of surgery, and before transplantation to decrease the risk of drop-out from the transplant waiting list, and to decrease the risks of recurrence in the long-term. *Downstaging* prior to resection is performed to render non-operable patients operable or to simplify the surgery, mainly for technical reasons. Finally, *downstaging* prior to transplantation is used as a selection tool to detect patients with low rates of recurrence among those that would be excluded according to recognized number-size criteria. While the present article is primarily exploring
the place of downstaging, we will also discuss neo-adjuvant options, as they help understanding the expected benefits of the various local HCC treatment modalities.

![Table of Neo-adjuvant treatments and Downstaging](image)

Figure 5.1: Definitions of downstaging and of neo-adjuvant treatments prior to resection or transplantation. In our opinion, the two words should not be used as synonyms.
Treatment of HCC prior to resection

When an HCC can be resected primarily, a pre-surgery neo-adjuvant treatment, like TACE, is usually not recommended (76). The main limitation is related to the time required to organize and perform TACE, which delays resection by 2-10 weeks and prevents up to 10% of patients from reaching surgery because of tumour progression or liver failure (77, 78). In addition, resection may be more challenging after TACE (requiring longer operative times, often in association with significant inflammatory reaction in the hilum and around the area of parenchymal treatment), TACE does not provide a measurable survival benefit, and has even been associated with increased mortality in two studies (77-82). This said, some of us do consider that one (and sometimes two) sessions of TACE should always be attempted prior to surgery, giving a chance of achieving tumour necrosis, which has been associated to higher rates of disease-free survival (80).

Some patients with good liver function do not qualify for primary resection because of the size and/or location of one or multiple HCCs, and may be considered for downstaging. Such a strategy has the potential to make surgery possible or easier (away from vascular structures), and potentially with decreased risks. With such a downstaging management, a limited number of non-resectable patients (6% to 28%) can subsequently undergo surgery (83, 84). Although high rates of recurrence have been observed (up to 40-85%), five-year survivals are between 25% and 60%, which is very reasonable considering the lack of alternative potentially curative options in these patients (80, 83-85).
place of downstaging as described above is relatively well accepted in the surgical community and does not require, in our opinion, further discussion apart from the best methods to obtain it.
Treatment of HCC prior to transplantation

The issues related to local HCC treatment prior to liver transplantation are more complex than those related to resection. In the setting of transplantation, these treatments will be considered differently whether a patient is within transplant criteria at presentation or not (neo-adjuvant vs. downstaging). The treatments will also be considered differently from a patient or community point of view, taking into account medical evidence-based data and ethical considerations:

Treatment of HCCs prior to liver transplant: neo-adjuvant vs. downstaging

Currently one third to one half of all HCC patients on the waiting list undergo local HCC treatment prior to transplantation (66, 86). The type of treatment varies from centre to centre, but TACE is the most frequently used, followed by RFA (66, 86-88).

Neo-adjuvant treatments (in contrast to downstaging) are primarily used to decrease the risk of drop-out from the waiting list (50, 64, 87, 89-92). They may be linked to a better post-transplant patient survival, as shown by a large UNOS-based study (78% with treatment vs. 74.8% with surveillance alone at two years, Risk Ratio=0.785, p=0.014) (88). This data is also supported by the observation that patients with full HCC necrosis after TACE have better post-transplant survivals than those with partial response (80, 93). Overall, a broader use of local neo-adjuvant HCC treatment in patients within transplant criteria appears justified (without delaying transplantation), as the risk of significant side-effects of these treatments is limited, with potential lower drop-out and higher survival rates.
A further argument in favour of local neo-adjuvant treatments is that they represent the best palliative option for patients who drop-out, avoiding the difficult situation of having delayed a proven effective treatment during the time spent on the waiting list.

When patients have HCCs beyond the accepted transplant criteria, the application of treatments aiming at downstaging tumours appears appropriate, as this is often the only hope of potential cure with a subsequent transplantation. In addition, tumour response to TACE could be used as a selection tool to help identify patients with an outcome that may be superior to that suggested by morphological criteria alone.

This strategy was initially suggested by the group in Hopital Paul Brousse, Paris, who retrospectively observed higher rates of survival in TACE responders than in non-responders in an analysis of patients with more than 3 nodules or nodules larger than 3 cm (80). Recognition and adoption of this strategy more widely has been slow because of poor agreement on definition, lack of selection criteria, absence of long-term outcome data and, until recently, the overall inability to construct prospective studies (exceptions listed in Table 5.1). To illustrate, the original report from UCSF on downstaging included only 16 months of median follow-up, too short to convincingly rule out the risk of HCC recurrence (this has been corrected in new studies from the same group) (94).
<table>
<thead>
<tr>
<th>Author</th>
<th>Journal, year</th>
<th>Evidence level**</th>
<th>Criteria to enter downstaging</th>
<th>Downstaging treatment (nb of patients)</th>
<th>Transplant criteria</th>
<th>Time stable prior to transplant</th>
<th>Downstaging success rate</th>
<th>Intent-to-treat post-HCC treatment survival</th>
<th>Post-transplant survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graziadei et al</td>
<td>Liver Transplant, 2003</td>
<td>13</td>
<td>Outside Milan, no vascular invasion, no extrahepatic disease</td>
<td>TACE</td>
<td>no limit</td>
<td>50% decrease in size</td>
<td>73%</td>
<td>31% (at 5 years)</td>
<td>41% (at 4 years survival)</td>
</tr>
<tr>
<td>Otto et al</td>
<td>Liver Transplant, 2006</td>
<td>15</td>
<td>Beyond Milan, no extrahepatic disease</td>
<td>TACE (62)</td>
<td>no limit</td>
<td>diameter of 5 target lesions</td>
<td>55%</td>
<td>?</td>
<td>74.5% (at 5 years)</td>
</tr>
<tr>
<td>Yao et al</td>
<td>Hepatology, 2008</td>
<td>16</td>
<td>1 lesion &gt;5 cm and ≤8 cm or 2 or 3 lesions at least 1 &gt;3 cm but ≤5 cm with total tumor diameter of ≤8 cm or 4 or 5 nodules all ≤3 cm with total tumor diameter ≤8 cm</td>
<td>TACE, RFA and/or resection (30)</td>
<td>Milan</td>
<td>minimum 3 months (mean: 6 months)</td>
<td>70%</td>
<td>69% (at 4 years)</td>
<td>92% (at 4 years DFS)</td>
</tr>
<tr>
<td>Ravaioli et al</td>
<td>AJT, 2008</td>
<td>19</td>
<td>1 lesion &gt;5 cm and ≤6 cm or 2 lesions at least 1 &gt;3 cm but ≤5 cm with total tumor diameter ≤8 cm or 4 or 5 nodules all ≤4 cm with total tumor diameter ≤12 cm</td>
<td>TACE, RFA, PEI and/or resection (48)</td>
<td>Milan and AFP≤400 ng/ml</td>
<td>minimum 3 months (mean: 6 months)</td>
<td>90%</td>
<td>62% (at 3 years)</td>
<td>71% (at 3 years DFS)</td>
</tr>
<tr>
<td>Chapman et al</td>
<td>Ann Surg, 2008</td>
<td>15</td>
<td>Beyond Milan, no lobar major vessel involvement or metastasis</td>
<td>TACE (76)</td>
<td>Milan</td>
<td>usually minimum 4 months (mean: 6 months)</td>
<td>31%</td>
<td>19% (at 3 years)</td>
<td>50% (at 5 years)</td>
</tr>
<tr>
<td>Lewandowski et al</td>
<td>AJT, 2009</td>
<td>18</td>
<td>T3</td>
<td>TACE (43)</td>
<td>Milan</td>
<td>no limit (mean: 11 months)</td>
<td>63%</td>
<td>84% (at 3 years)</td>
<td>78.8% (survival at 3 years)</td>
</tr>
<tr>
<td>De Luna et al</td>
<td>AJT, 2009</td>
<td>16</td>
<td>Beyond Milan, no lobar major vessel involvement or metastasis</td>
<td>TACE (386)</td>
<td>Milan</td>
<td>no limit (median: 2 months)</td>
<td>41.5%</td>
<td>25% (at 5 years)</td>
<td>66% (at 5 years DFS)</td>
</tr>
<tr>
<td>Jang et al</td>
<td>Aliment Pharmacol Ther, 2009</td>
<td>11</td>
<td>Beyond Milan, no lobar major vessel involvement or metastasis</td>
<td>TACE (89)</td>
<td>Milan</td>
<td>minimum 6 months</td>
<td>41.5%</td>
<td>25% (at 5 years)</td>
<td>66% (at 5 years DFS)</td>
</tr>
</tbody>
</table>

**Proposed strategy**

TTV≤250 cm³

| TTW≤15 cm³ | open*** |

RFA: radio-frequency ablation, TACE: transarterial chemo-embolisation, TACI: transcatheter arterial chemoinfusion, TARE-Y90: transarterial radioembolization with Yttrium-90 microspheres

T2:T3: 1 nodule ≤5 cm or up to 3 nodules with one ≤3 cm

* referenced in Medline until Oct. 25, 2009 under "liver transplant, hepatocellular carcinoma, downstaging"

** assessed according to the Downs and Black checklist (51)

*** treatment is guided by tumor and patient characteristics
More recent reports have demonstrated that downstaging can be successful in 24 to 90% of patients (Table 5.1). This wide range of observed rates is primarily related to the use of different criteria to include patients in downstaging protocols and different criteria to subsequently decide on listing for transplantation. Some groups consider patients for listing as soon as HCCs have decreased in size by 30 or 50%, while others will require full necrosis (absence of any uptake on CT) prior to doing so (71, 92, 95, 96). In addition, some centres follow Milan transplant criteria, while others use expanded ones (71, 92, 95-97).

Despite these limitations, recent prospective studies have demonstrated that downstaging is a valid strategy prior to transplant (71, 96, 98): following successful downstaging, post-transplant disease-free survivals have been reported at over 70% at 3 years, and intention-to-treat post-HCC treatment survivals between 60 and 70% at 3 years (71, 92, 96, 98). Such outcomes have been substantially better than anticipated in a group of patients with such an advanced cancer, in some series not just beyond Milan criteria, but beyond UCSF criteria as well (75, 99). In addition, they appear to compare favourably with the generally accepted minimal long-term post-transplant survival of 50% at 5 years, unrefined and arbitrary but consensual target (100, 101).

For these reasons, it appears legitimate to attempt downstaging in any patient beyond transplant criteria and without distant metastasis, even more so as downstaging treatments are identical to palliative ones. The downside of a too liberal access to downstaging strategies (and subsequent transplant) could be an enhanced competition for donor livers with patients within standard transplant
criteria (with or without HCC) and should be countered by defining reasonable inclusion criteria.

*Which criteria should be used to include patients in a downstaging protocol?*

While any patient with HCC beyond transplant criteria, but without distant metastasis, may benefit from a local HCC treatment (palliative or downstaging), we believe that only clearly selected candidates should enter downstaging protocols. The two main reasons for a strict attitude are: a) the need to gather robust data on this topic, and b) the implicit obligation to treat all patients on the waiting list equitably, including those with HCC or benign disease. This even if from a patient's point of view transplantation may represent the best option for cure. The individual's perspective that a small chance of successful transplantation is better than the certainty of HCC progressing on palliative treatment has to be balanced with the societal demand - and transplant program commitment - for the responsible use of a scarce resource.

To establish a reliable selection policy, three points have to be taken into account:

a) defined entry criteria

- size/number or total tumour volume of HCC
- biological/pathological and molecular markers

b) defined end-points of successful downstaging

- radiological
i. degree of necrosis
ii. decrease in size

- biological: AFP

c) defined time between downstaging and listing for transplant

**Defined entry criteria**

The criteria to enter a downstaging program should include patients who have well defined and acceptable chances of good outcomes after transplantation if the downstaging goal is reached. Such a strict attitude would maintain the expansion of transplant criteria within reasonable limits, and allow gathering of robust and comparable data for progress. For this reason patients with metastasis or with large vessel thrombosis seen on radiology should be excluded. Several groups have prospectively assessed various scores (Table 1), including UNOS T3 (1 nodule >5 cm or up to 3 nodules with one >3 cm) or combinations of size and number with up to 5 nodules and a total tumour diameter of 8 or 12 cm (71, 96, 98). While these scores have not been validated externally, they appear reasonable as they can lead to post-transplant disease-free survival rates over 70% at 3 years (71, 96, 98). We would however advocate that the UNOS T3 is too restrictive regarding tumour number, as patients with more than 3 lesions (even of small size) cannot be considered for downstaging. Following our previous work on Total Tumour Volume (TTV) (66, 86), the group in Edmonton has decided to include for downstaging all patients with TTV ≤250 cm³. This corresponds to a single HCC of 7.8 cm in diameter or 3 HCCs of 5.4 cm...
cm, but any size and number combination can be considered as long as the cumulated tumour volume remains within the limit. TTV does not include any number restriction and has better expected radiological accuracy (larger HCCs have more weight in the score and can be better defined by radiology), but downstaging results are still pending (66).

Evidence is accumulating that biological markers such as alpha-fetoprotein (AFP) or PIVKA-II add additional predictive accuracy if used in addition to morphological characteristics (13, 86, 96, 102). We can speculate that AFP and PIVKA-II provide a good assessment of tumour biology, including microvascular invasion, grade and tumour aggressiveness in general. In a Scientific registry for Transplant Recipients (SRTR)-based study, we have shown that tumour volume and AFP are independent predictors of post-transplant survival, and that morphological criteria alone will miss many patients with expected poor outcomes (86). Even within Milan criteria, AFP values > 400 ng/ml were able to select patients at high risk of tumour recurrence.

**Defined end-points of successful downstaging**

While it is clear that patients not responding to downstaging should not be considered for transplantation (92, 97), transplant criteria after successful downstaging remain to be defined more precisely. We believe the most useful parameters for this are radiological response in terms of viability and size of the tumours, and probably biological response measured as a decrease in AFP.
While some investigators have accepted for transplantation patients with HCCs demonstrating partial response (decrease in size of 30% to 50%), the most recent studies have considered only patients whose tumours have demonstrated complete ablation/no augmentation on imaging (71, 92, 95, 96). Intuitively, this attitude makes sense as extinction of vascularisation after treatment can be taken as a surrogate marker for a favourable biology, while on the contrary the probabilities of distant spread associated with a large tumour size likely have not changed if a lesion is still partially viable.

Lesions that are fully inactive on radiology are no longer counted as a nodule in the final score in most studies (71, 96).

As for the final radiological endpoint to define successful downstaging, most published studies have been using the goal of Milan criteria after treatment (with or without AFP ≤400 ng/ml) to select patients eligible for transplantation (Table 1)(71, 96-98, 103, 104). Results have been similar to those achieved in patients within Milan from the beginning (103).

As for biological markers, a persistently high AFP after treatment should raise the suspicion of distant spread or vascular invasion and may represent a useful marker of unsuccessful downstaging.

**Defining a time between downstaging and listing for transplant**

The time interval between downstaging and transplantation can be considered an additional tool to help in selection of HCCs with favourable biology. This “test of time”, will disclose rapidly recurring lesions, vascular invasion and distant
metastases. Some published reports have not pre-defined a minimal surveillance time, but this was achieved in fact naturally, by a mean waiting time of at least 6 months between activation on the waiting list after downstaging and the date of transplantation (Table 5.1). The commonest surveillance time in published reports was 3 months, and appears as the minimum required. In contrast, centres allowing transplantation early after downstaging may face a shift from progression on the waiting list to recurrence in the post-transplant period, and thereby experience poor overall results (105).

In general, the criteria for inclusion in downstaging protocols used to date (with an upper limit at 8 cm, or 250 cm$^3$), while still needing external validation within formal protocols, appear reasonable, and have proven to be working. As a measure of achievement of successful downstaging we would recommend the endpoint of Milan criteria, counting fully inactive nodules as non-existent and partially inactive nodules at their original size. We would also suggest excluding patients with an AFP remaining above 400ng/ml after treatment, and a minimum observation time of 6 months between entry into the downstaging program and activation on the waiting list for transplantation. Additional data forthcoming in future may validate more expanded criteria, or a shorter surveillance period.
Which local HCC downstaging treatment should be used?

The choice of a downstaging treatment should be based both on the morphological characteristics of the HCC and on the patient condition, balancing the risks and benefits of each technique. The efficiency of some treatments (including TACE and TARE) is linked to HCC biology, with a better response in HCCs with higher blood supply and uptake. Others are physico-mechanical treatments, like RFA, where an HCC can be destroyed whether well differentiated or not.

Radiofrequency ablation (RFA)

RFA uses radiofrequency energy for hyperthermic ablation. It can be performed by interventional radiology or at laparoscopy. Over time, it has replaced ethanol injection in the treatment of small HCCs in most centres, as RFA ablation results in a higher rate of complete necrosis (usually over 90%) and requires fewer treatment sessions (106-110). RFA is safe in terms of liver function and can be performed even in cases of advanced liver failure (111, 112). It is most effective for the treatment of HCCs ≤3cm in diameter (Table 5.2). RFA should be avoided for lesions located close to the surface of the liver and neighbouring organs due to the risk of rupture of the liver capsule and seeding of malignant cells in the peritoneal cavity. Another potential complication of RFA (again shared with ethanol ablation or biopsy) is the seeding of cancer along the needle tract, which has been estimated to occur in 1-2% (87). In addition, the use of RFA can be hampered by the presence of ascites, which should first be drained. In the
absence of transplantation, 5-year survivals up to 30-40% can be expected after RFA (106, 108), limited mainly by liver failure and the development of new primaries.

<table>
<thead>
<tr>
<th>Indications</th>
<th>Risks</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiofrequency ablation</td>
<td>small HCC (usually ≤3 cm) away from the liver surface away from major vessels</td>
<td>HCC seeding (1-2%) liver rupture (small)</td>
</tr>
<tr>
<td>Transarterial chemoembolisation</td>
<td>any HCC size preserved liver function (Child A-B) uptake of contrast</td>
<td>liver failure arterial injury (2%)</td>
</tr>
<tr>
<td>Transarterial radioembolisation</td>
<td>any HCC preserved liver function (Child A-B) absence of intra-hepatic shunt uptake of contrast</td>
<td>off-target embolisation arterial injury</td>
</tr>
</tbody>
</table>

Table 5.2: Benefits and limitation of local HCC treatments

Transarterial embolisation (TACE)

TACE is currently the most popular neoadjuvant treatment option for patients with HCCs (60, 71, 80, 92). Treatment is usually performed using a combination of mitomycin, Adriamycin or cisplatinum mixed with lipiodol as the drug carrier and an embolization using permanent or re-absorbable occlusive particles (gelatine). Post-procedure the patients are hospitalised for observation (usually for 24 hours).

Several randomized studies have compared TACE to conservative management in HCC patients not candidate to curative options, and when analysed together in a meta-analysis, TACE demonstrated a significant superiority in terms of survival (113). TACE also allows treatment of larger tumours than RFA and may simplify treatment of patients with multiple tumours. On the basis of the studies quoted
above, we suggest TACE may merit consideration as the first line neo-adjuvant option prior to resection or transplantation in the majority of patients.

The main risks of TACE are linked to the ischemic insults of the embolisation. Patients with large lesions may develop a postembolisation syndrome due to tumour necrosis, with fever and abdominal pain. When a large area of liver parenchyma has been embolised, patients are also exposed to the risk of liver failure, and TACE should as a rule not be attempted in patients with decreased liver function (Child-Pugh C), except when an hyper-selective TACE can be offered by expert hands. Finally, this procedure includes a small risk of arterial injury, estimated at 2% (114). Another limitation is linked to the poor uptake of dye by hypo-vascular HCCs, and these lesions may be better treated with RFA.

*Transarterial radioembolisation (TARE)*

TARE is a transarterial procedure, which is performed by embolising 20 to 30 μm insoluble glass microspheres impregnated with yttrium-90 (a β emitter) (TheraSphere, MDS Nordion, Ottawa, Canada; SIR-Spheres, Sirtex Medical Limited, Australia)(115). The treatment induces a local necrosis of the tumour, due to the β emission. This type of local HCC treatment has gained more interest in the recent years. It represents an interesting alternative to TACE, as it appears to induce a more efficient decrease in tumour size, with a shorter time to response (4.2 vs. 10.9 months)(98). In addition, TARE can be performed in cases of portal vein thrombosis, a contraindication to TACE. Finally, TARE induces a regeneration of
the contra-lateral liver, which may prove to be useful in case of planned resection with a small liver remnant (116).

TARE is well tolerated in most patients leading to a discharge within 2 to 6 hours (without inpatient surveillance like TACE). The most common side-effects include fatigue and flu-like symptoms (98). Several complications of TARE are similar to those of TACE, including the risk of arterial injury (117). The presence of excessive intrahepatic shunting should be excluded by 99Tc-macroaggregated albumin scanning and mesenteric angiogram to minimize the risk of nontarget embolization, especially radiation injury to the lungs (98). While TARE has several advantages over TACE, its place remains to be fully defined (together with its cost-efficiency)(98).

Altogether, the choice of a local HCC treatment should be guided by the tumour and patient characteristics, as well as the local expertise. In consideration of the relative advantages and risks of ablative and trans-arterial approaches, we would suggest primary application of RFA for more centrally placed tumours when < 3 cm diameter and in candidates with poor liver function who may not tolerate transarterial therapies well. TACE or TARE should receive primary consideration in candidates with satisfactory liver function when tumours are above optimal size for RFA (>3cm), when multiple tumours are present or when tumours are in a subcapsular position or adjacent to major vessels or bile ducts. Incomplete control should lead to re-evaluation and consideration of the alternative approaches.
Final considerations

While further validations to refine the application of downstaging strategies are required, most of them appear reasonable, and can be used with advantage in patients with primarily unresectable tumours before partial hepatectomy, or to identify patients with a high likelihood of a good outcome despite being outside current transplantation criteria. Current evidence suggests that patients with solitary HCCs up to 8 cm in diameter and with up to 5 tumours (all ≤4 cm with total tumour diameter ≤12 cm) can be considered for downstaging. We would favour combining these cut-offs within the Total Tumor Volume score (250 cm$^3$), or with the up to 7 criteria recently published by the Metroticket collaborative study group (67, 86). Patients who reach traditional Milan criteria (inactive tumours counting as zero), with no contraindication to transplantation appearing during a waiting time of at least 3 months (ideally 6 months) have a more favourable biology, as shown by a low recurrence rate. These criteria may be further refined by adding AFP as a selection marker, and should be confirmed in further prospective investigations that can be organised in the present collaborative spirit.

Acknowledgements

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References


6. Sirolimus-based immunosuppression: benefits and side-effects

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by Christian Toso, Glenda A. Meeberg, David L. Bigam, Jose Oberholzer, A. M. James Shapiro, Klaus Gutfreund, Mang M. Ma, Andrew L. Mason, Winnie W. S. Wong, Vincent G. Bain, Norman M Kneteman
Abstract

We report long-term outcomes and side effects after transplantation for hepatocellular carcinoma (HCC) using de novo, sirolimus-based immunosuppression (IS).

A total of 70 patients with HCC (mean age: 54.4+/7 years, female/male: 12/58) were transplanted and included in the study. Immunosuppression included de novo sirolimus, low-dose calcineurin inhibitor for 6 to 12 months, with short-course (3 months) or no steroids.

After 49 months-median follow-up, eight patients have experienced an HCC recurrence, 2 of 34 when Milan criteria were respected (6%) and 6 of 36 when beyond Milan criteria (17%). One- and 4-year tumor-free survivals were 85 and 73%, when Milan criteria were respected and 82% and 75% when they were not, respectively. (P=0.9). After recurrence, mean survival was 23+/28 months. Half (35 of 70) of the patients experienced a rejection. Incisional hernia (24 of 70, 34%), wound infection (12 of 70, 17%), anemia (39 of 70, 56%), leucopenia (39 of 70, 56%), high triglyceride (43 of 70, 61%), and cholesterol (28 of 70, 40%) levels and mouth ulcers (20 of 70, 29%) were among the most frequent complications. No hepatic artery thrombosis was observed.

These data suggest that de novo sirolimus-based immunosuppression is associated with satisfactory outcomes after transplantation, even in selected patients beyond Milan criteria. The protocol has proven safe, with an acceptable side-effect profile. This study supports the conduct of larger randomized trials investigating sirolimus after transplantation for HCC.
Introduction

Sirolimus based immunosuppression is associated with a reduced risk of de novo malignancy after transplantation (1). An anti-cancer impact of sirolimus was suggested in the mid 1980’s (2) and has been further supported by recent data. Sirolimus can prevent angiogenesis by interfering with VEGF-mediated pathways in endothelial cells, thus limiting the growth of tumors (3). It impacts on established tumor vessels, by inducing extensive microthrombi (4) and can inhibit the growth of human hepatoma cells in vitro (5).

While these data suggest clear anti-cancer properties, it remains to be determined whether sirolimus can reduce the risk of post-transplant recurrence in patients with hepatocellular carcinoma (HCC). While no randomized study is available to date, published studies (including our own experience) have demonstrated the successful use of sirolimus from the time of transplantation for HCC (6-8). These preliminary studies have reported on limited numbers of patients and relatively short follow-up. The present series investigates the long-term outcome of de novo sirolimus-based immunosuppression after transplantation for HCC in an expanded group of 70 patients with follow up now extending as long as 10 years. We also report side effect profile with this sirolimus-based immunosuppression protocol.
Patients and Methods

Inclusion criteria

From December 1996 to March 2006, all liver recipients documented to have HCC pre-transplant or with HCC discovered on the explanted liver were prospectively enrolled in this pilot study exploring sirolimus-based immunosuppression. This protocol was approved by the Health Research Ethics Board at the University of Alberta and was supported by the Canadian Liver Transplant Study Group.

HCC patients with a single tumor up to 7.5 cm in diameter or multiple tumors up to 5 cm in diameter (without numerical restriction) were considered for transplantation. Contraindications included extrahepatic disease or major vascular invasion on imaging. Candidates with tumors over 5 cm diameter required preoperative biopsy to rule out high tumor grade, which in combination with tumor size >5cm was considered unacceptable for transplant.

Immunosuppression (Table 6.1)

Sirolimus was initially given at 0.1 mg/Kg/day to achieve trough levels between 15 and 20 μg/L as tolerated (Rapamune, Wyeth Research Laval, Quebec, Canada). The first 10 patients received cyclosporine (Neoral, Novartis, Laval, Quebec) with target C0 levels of 150 μg/L, and the 56 following received tacrolimus (Prograf, Astellas, Markham, Ontario, Canada) with target trough levels between 3 and 6 μg/L. The first 21 patients received steroids for three months, including 500 mg intra-operative methylprednisolone and a rapid
tapering to 20 mg/day at day 5. The aim was to wean steroids off by month 3 in rejection-free patients. In the subsequent 45 patients, steroids were replaced with Daclizumab induction (Zenapax, Hoffman-La-Roche, Mississauga, Ontario, Canada). In all rejection-free patients, the aim was to further decrease and wean calcineurin inhibitors between 3 and 6 months post-transplant, to achieve single-drug sirolimus maintenance immunosuppression with target trough levels between 12 and 15 μg/L.

<table>
<thead>
<tr>
<th>Initial IS</th>
<th>One year (%)</th>
<th>Four years (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirolimus</td>
<td>19 (37)</td>
<td>19 (70)</td>
</tr>
<tr>
<td>Sirolimus + tacrolimus</td>
<td>19 (37)</td>
<td>2 (7.5)</td>
</tr>
<tr>
<td>Sirolimus + cyclosporine</td>
<td>3 (6)</td>
<td>-</td>
</tr>
<tr>
<td>Sirolimus + steroids</td>
<td>2 (4)</td>
<td>-</td>
</tr>
<tr>
<td>Sirolimus + mycophenolate mofetil</td>
<td>3 (6)</td>
<td>-</td>
</tr>
<tr>
<td>Sirolimus + cyclosporine + steroids</td>
<td>10 (14)</td>
<td>-</td>
</tr>
<tr>
<td>Sirolimus + tacrolimus + steroids</td>
<td>11 (16)</td>
<td></td>
</tr>
<tr>
<td>Sirolimus + tacrolimus + daclizumab</td>
<td>49 (70)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IS: immunosuppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%) : pourcent of surviving patients having achieved specified follow-up</td>
</tr>
</tbody>
</table>

Table 6.1: Immunosuppression combinations
Infection prophylaxis and treatment

Post-operative prophylaxis included 1g iv cefatoxime three times a day for 48 hours, Nystatin 500,000 units/day until discharge and co-trimoxazole (trimethoprim/sulfamethoxazole) 400 mg/800 mg daily for the first six months. After 1999, CMV prophylaxis was given in case of mismatch (donor+/recipient-). It included 1g po ganciclovir three times daily or as appropriate according to renal function for a total of 14 weeks. Patients with HBV received post-transplant prophylaxis with lamivudine ± hepatitis B immunoglobulin. No prophylaxis was administrated to HCV patients. Subsequent to reports of potential increase in risk of hepatic artery thrombosis with early sirolimus therapy, patients in the sirolimus group received a five day treatment with 300 U/hour iv heparin and 81 mg po aspirin daily thereafter.

Follow-up, side-effects definition and statistical analysis

Mortality, recurrence and the rate of ongoing sirolimus treatment were recorded over a median follow-up of 49 months (ranges 6.5-119). Side effects are reported for the first year after transplant, or as subsequently specified. Anemia was defined as hemoglobin under 8 g/dL for at least one week or requirement for treatment. Leucopenia was defined as leucocyte counts under 3 x10^9/L for at least 5 days or requirement for G-CSF therapy. Dialysis included all patients requiring dialysis between the first and the twelfth months post-transplant. Creatinine levels were studied up to 5 years after transplantation. Lipid-lowering and blood pressure lowering drugs included patients with
increased or modified treatment. Incisional hernias were reviewed for the entire follow-up period and included hernias secondary to the transplant incision and recurrence after repair. Delayed wound healing was defined as a wound still requiring nursing therapy one month after surgery.

Data were prospectively collected in an electronic database (OTTR, Hickman-Kenyon Systems, Omaha, NE). They were subsequently completed by phone interviews of living patients. Analysis was performed retrospectively.

Survivals were analyzed by the Kaplan-Meier method and differences between groups were further tested by the log-rank test. Tumor-free survival was defined as the absence of tumor recurrence and of death (related to HCC or not). Analysis was also performed by Chi-square or Student T tests, when applicable.

Calculations used Microsoft Excel (Microsoft Corp, Redmond, WA) and Statistica (Statsoft, Berikon, Switzerland) softwares.
Results

HCC recipient and tumor characteristics

During the study period, 517 adult patients received 547 liver transplants. Among them, 70 had an HCC and were included in the study. Two liver recipients had incidental HCCs within Milan criteria and were not included in the present study, because of previous enrollment in other trials. Fourteen patients were withdrawn from candidacy while on the waiting list due to tumor progression. Three were excluded due to tumor of > 5cm that was poorly differentiated on biopsy (two prior to and one after listing). Fifty-six patients died on the waiting list, including one with HCC.

The study population included 12 females and 58 males, with a median age of 53 years (range 37-67; Table 6.2). All received whole cadaveric liver grafts, none were retransplants. The most frequent underlying liver diseases were related to hepatitis C virus infection (HCV), hepatitis B virus infection (HBV) and alcoholic cirrhosis (Table 6.2).
<table>
<thead>
<tr>
<th>demographic</th>
<th>count/percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>53 (range 37-67)</td>
</tr>
<tr>
<td>Gender</td>
<td>female:12/ male:58</td>
</tr>
<tr>
<td>Race (%)</td>
<td>Asian 14 (20)</td>
</tr>
<tr>
<td>Cause of liver disease (%)</td>
<td>HCV (±alcohol, ±HBV) 34 (49)</td>
</tr>
<tr>
<td></td>
<td>HBV 16 (23)</td>
</tr>
<tr>
<td></td>
<td>Alcohol 8 (11)</td>
</tr>
<tr>
<td></td>
<td>Cryptogenic 5 (7)</td>
</tr>
<tr>
<td></td>
<td>Primary biliary cirrhosis 4 (6)</td>
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<td></td>
<td>Hemochromatosis 2 (3)</td>
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<tr>
<td></td>
<td>Primary sclerosing cholangitis 1 (1)</td>
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<td></td>
<td>Autoimmune 0</td>
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<td>Other 0</td>
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<td>Raw MELD score (%)</td>
<td>&lt;10 33 (47)</td>
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<tr>
<td></td>
<td>≥10; &lt;20 23 (33)</td>
</tr>
<tr>
<td></td>
<td>≥20 14 (20)</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus infection
HBV: hepatitis B virus infection

Table 6.2: Demographics and patient characteristics
Fifty-one patients had known HCC prior to transplant (73%) while HCC was first discovered on the explanted liver in 19 recipients (27%). One third of the patients had pre-transplant HCC treatment (Table 6.3), including ethanol injection (12, 17%), trans-arterial chemoembolisation (5, 7%), surgical resection (3, 4.5%) and radiofrequency ablation (3, 4.5%). Pretransplant treatment was performed in 8/34 patients within Milan criteria and 16/36 beyond Milan criteria (p: 0.2).

While 43% of patients had α-fetoprotein levels lower than 10 ng/mL, 11% had levels over 1000 (Table 6.3).

A total of 181 tumors could be identified in the explanted livers (Table 6.3). Up to 20 tumors per liver were found, but most had not more than 3; mean number was 2.8 ±2.7. While the majority of tumors were ≤ 3 cm; ten tumors were greater than 5 cm in diameter, and tone patient was found to have a 12.5 cm diameter tumor on explant. This patient had undergone a CT revealing a 7.8 cm tumor while on the waiting list, but was not removed from the list in a timely fashion and proceeded to transplant.

Based on pathology, 34 patients (49%) fulfilled Milan criteria, with one tumor up to 5 cm or up to three tumors ≤3 cm each (9)(Table 6.3). Forty-nine patients (70%) fulfilled UCSF criteria, with one tumor up to 6.5 cm, or up to 3 tumors with the largest ≤4.5 cm and a total diameter ≤8 cm (10). Patients were also classified according to the 6th edition of the TNM classification (11). Twenty-three (33%) were of stage I (single tumor ≤5 cm without vascular invasion), 40 (57%) of stage II (single tumor ≤5 cm with vascular invasion or multiple tumors, none >5 cm) and 7 (10%) of stage III (multiple tumors with any >5 cm or major vessel invasion). No
patients whose tumors has progressed to major vessel invasion on preoperative imaging were listed for transplant.

Although patients with HCC larger than 5 cm underwent a protocol biopsy, in order to exclude individuals with large and poorly differentiated cancers, 17 (25%) of patients had poorly differentiated HCC on explant pathology (most under 5 cm).

When analyzed based on pre-transplant radiology, 51 patients were known to have HCC. Thirty-two patients (63%) fulfilled Milan criteria and 19 did not (37%). Forty-one patients (80%) fulfilled UCSF criteria and 10 did not (20%) by preoperative radiological assessment.
<table>
<thead>
<tr>
<th>Pre-transplant treatment (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol injection</td>
<td>12 (17)</td>
</tr>
<tr>
<td>TACE</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Surgical resection</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>Radiofrequency ablation</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>None</td>
<td>47 (67)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>α-fetoprotein levels (ng/mL) (% out of 65 patients tested)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>28 (43)</td>
</tr>
<tr>
<td>≥10; &lt;100</td>
<td>21 (32)</td>
</tr>
<tr>
<td>≥100; &lt;1,000</td>
<td>9 (14)</td>
</tr>
<tr>
<td>≥1,000; &lt;10,000</td>
<td>7 (11)</td>
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<table>
<thead>
<tr>
<th>Tumor size (% out of 181 tumors)</th>
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<tr>
<td>&lt;1 cm</td>
<td>48 (27)</td>
</tr>
<tr>
<td>≥1 cm; &lt;2 cm</td>
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<td>≥4 cm; &lt;5 cm</td>
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<tr>
<td>≥5 cm; &lt;7 cm</td>
<td>7 (4)</td>
</tr>
<tr>
<td>≥7 cm</td>
<td>3 (2)</td>
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<tr>
<th>Number of tumor (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>30 (43)</td>
</tr>
<tr>
<td>2</td>
<td>14 (20)</td>
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<tr>
<td>3</td>
<td>8 (11)</td>
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<td>4</td>
<td>7 (10)</td>
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<td>5</td>
<td>6 (9)</td>
</tr>
<tr>
<td>6</td>
<td>3 (4)</td>
</tr>
<tr>
<td>≥7</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

| Milan criteria respected (%)                            | 34 (49) |
| MCSF criteria respected (%)                             | 49 (70) |

<table>
<thead>
<tr>
<th>Stage (%)</th>
<th></th>
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<tbody>
<tr>
<td>I</td>
<td>23 (33)</td>
</tr>
<tr>
<td>II</td>
<td>40 (57)</td>
</tr>
<tr>
<td>III</td>
<td>7 (10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade (% out of 67 cases)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16 (24)</td>
</tr>
<tr>
<td>II</td>
<td>34 (51)</td>
</tr>
<tr>
<td>III</td>
<td>17 (25)</td>
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</table>

<table>
<thead>
<tr>
<th>Microvascular invasion (% out of 68 patients)</th>
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</thead>
<tbody>
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<td>35 (51)</td>
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<table>
<thead>
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<th>Incidental tumor (%)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>19 (27)</td>
</tr>
</tbody>
</table>

TACE: trans-arterial chemoembolisation
Milan criteria as previously defined by Mazzaferro et al (9)
UCSF criteria as previously defined by Yao et al. (10)
Grade I: well differentiated, grade II: moderately differentiated, grade III: poorly differentiated

Table 6.3: Patient and histological tumor characteristics
Survival, tumor recurrence

Overall survival was 85 and 77% at one and four years. Eight patients experienced an HCC recurrence (11.4%, Table 6.4), from 4 to 52 months after transplant (mean 17 ±10 months). Three occurred within the first year and the other five from the first to the fourth year after transplantation. Recurrences were homogeneously distributed over the study period and no impact of the variations of the sirolimus-based immunosuppression could be identified. Two were in patients having undergone chemo-embolization and one after alcohol ablation. Five recurrences appeared in patients with tumors demonstrating microscopic vascular invasion (5/35, 14%); while 6 occurred in patients that carried poorly differentiated HCC (6/17, 35%). Survival free of tumor recurrence was 84 and 74%, at one and four years. Mean survival after recurrence was 23 ± 28 months, without subsequent non-surgical HCC treatment, but with ongoing sirolimus therapy. Two patients underwent resection of recurrent tumors.
# Table 6.4: Recurrences

<table>
<thead>
<tr>
<th></th>
<th>Total (%)</th>
<th>Milan classification</th>
<th>UCSF classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Respected (%)</td>
<td>Not respected (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (6)</td>
<td>6 (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (10)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Mean time from transplant to recurrence (months ± SD)</td>
<td>17 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean survival after recurrence (months ± SD)</td>
<td>23 ± 26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Eighteen patients died during the follow-up. Five deaths were related to HCC recurrence. One patient with an asymptomatic HCC recurrence died of myocardial infarct.

Based on pathology, Milan and UCSF failed to significantly discriminate patient outcome (p: 0.9 both, Figure 6.1). One and four year tumor-free survivals were 85 and 73% when Milan criteria were fulfilled and 82 and 75% when they were not. Similarly, one and four year tumor-free survivals were 84 and 77% when UCSF criteria were fulfilled and 84 and 72% when they were not.

According to the pre-transplant radiological staging, both Milan and UCSF classifications failed to predict tumor-free survival (p: 0.9 and 0.8). One and four year tumor-free survivals were 86 and 75% when Milan criteria were fulfilled and 78 and 70% when they were not. Similarly, one and four year tumor-free survivals were 85 and 73% when UCSF criteria were fulfilled and 80% and 80% when they were not. Using the pre-operative radiological imaging for classification, 5 patients of 32 within Milan criteria experienced a recurrence (16%) while 3 of 19 beyond Milan criteria (16%) were associated with recurrence (p: 0.9).

Only 53% of patients were correctly classified by pre-transplant imaging as meeting Milan criteria. Of the 32 patients fulfilling Milan criteria on radiology, 20 (63%) were beyond on pathology, most due to pathological findings of increased numbers of small tumors.
Figure 6.1: Tumor-free survival, according to Milan (A) and UCSF (B) criteria. P values were 0.9 for both classifications (Log-rank test). The number of patients at risk at each time-point for each group is shown under the X-axis.
Sirolimus treatment tolerance

The aim of the immunosuppressive protocol was to achieve long-term sirolimus single-drug immunosuppression, but treatment could be modified according to tolerance and side effects. One and four years after transplant, 88 and 80% of the patients were still on sirolimus (cumulative number of patients alive and on sirolimus, Figure 6.2). Most patients could over time achieve sirolimus single-drug immunosuppression (70% at four years, Table 6.1). Mean level of sirolimus, given alone or in combination with other drugs, was 14.7 ±6 and 10.2 ±4 at one and four years post-transplant. All patients with HCC recurrence were still on sirolimus.

Sirolimus therapy had to be discontinued because of hematological toxicity (n=4), wound healing problems (n=3), mouth ulcers (n=2), dyslipidemia (n=1) and other (n=3).
Figure 6.2: Number of patients alive and still on sirolimus treatment after transplantation. The number of patient at risk at each time-point is show under the X-axis.
Rejections and sirolimus-associated side effects

Thirty-five patients (50%) experienced 53 rejections (Table 6.5). No hepatic artery thromboses occurred; but three hepatic artery stenoses were noted, and treated by angioplasty or surgical revision. Bacterial infections included mainly pneumonia, bacteremia, pseudomembranous colitis and intra-abdominal infection. Viral (except HCV recurrence) and fungal infections occurred in 29 and 4% of patients. A biopsy-proven HCV recurrence has been observed in 18 patients (53%). Two of them died of pneumonia and of chronic liver failure.

The main hematological disorders included anemias and leucopenias (Table 6.5). Forty-three and 23% of patients required iron or erythropoietin treatment. Dyslipidemia was also a frequent problem, with 31% of patients requiring modification or increase of their lipid-lowering medications. Patients on dialysis, newly requiring insulin and requiring increased blood pressure lowering therapy reached 3, 17 and 24%. Creatinine levels demonstrated a trend to increase, especially within the first year post-transplant (prior to transplant: 104 ±51 μmol/L (1.2 ±0.6 mg/dL), at 6 months: 123 ±57 μmol/L (1.4 ±0.6 mg/dL), p: 0.06; 12 months: 136 ±91 μmol/L (1.5 ±1 mg/dL), p: 0.03; 3 years: 114 ±41 μmol/L (1.3 ±0.5 mg/dL), p: 0.3 and 5 years: 133 ±61 μmol/L (1.5 ±0.7 mg/dL), p: 0.1; p values compare the level at the specific time point with the one at baseline.

Wound healing was delayed by sirolimus; 14% of patients had wounds that were not completely re-epithelialized one month after surgery (Table 6.5). One third of patient also experienced incisional hernias. Twenty patients experienced mouth
ulcers related to sirolimus. Edema, dermatitis, joint pains and pleural effusions were rare events.
Patients with rejection 35 (50)
Patients with biopsy-proven rejection 34 (49)
Steroid-resistant rejection 1 (1.5)

Surgical complications
Biliary complications 19 (27)
Hepatic artery stenosis 3 (4)
Hepatic artery thrombosis 0
Portal vein thrombosis 0

Infections
Pneumonia 14 (20)
Bacteriemia 8 (11)
Pseudomembranous colitis 7 (10)
Intraabdominal abscess/ Peritonitis 6 (8.5)
Other bacterial infections 4 (5.5)

HZV 1 (1.5)
HSV I 13 (19)
HSV II 2 (3)
CMV 4 (6)
Fungal infections 3 (4)

Hematological disorders
Anemia 39 (56)
Treatment with iron 30 (43)
Treatment with erythropoietin 16 (23)
Leucopenia 39 (56)
Treatment with G-CSF 10 (14)

Other
Dialysis 2 (3)
Hypertriglyceridemia 43 (61)
Hypercholesterolimia 28 (40)
Lipid-lowering drug introduced 22 (31)
Blood pressure lowering drug introduced 17 (24)
Insulin treatment introduced 12 (17)

Wound infection 12 (17)
Delayed wound healing 10 (14)
Incisional hernia 24 (34)

Mouth ulcers 20 (29)

* wound infections excluded

Table 6.5: Number of patients alive and still on sirolimus treatment after transplantation. The number of patient at risk at each time-point is show under the X-axis.
Discussion

This trial of liver transplantation in HCC patients suggests that de novo sirolimus-based immunosuppression is associated with acceptable long-term outcomes, even in selected patients beyond Milan criteria. The predominant side-effects include incisional hernia, anemia, leucopenia, dyslipidemia and mouth ulcers. These data require confirmation by a randomized study, which may be guided by the present pilot experience.

Sirolimus has been clearly demonstrated to possess experimental anti-cancer properties in both in vitro and in vivo studies (12). To date, no randomized study is available that reports its impact on patients with HCC. Accordingly, the present results must be interpreted with care. The present data do however, suggest some potential benefit of sirolimus on HCC after liver transplant.

First, the HCC recurrence rate of patients within Milan (6%) or beyond UCSF (14%) criteria are among the lowest reported, with other studies ranging from 5 to 10.5% (13-15) and 25 to 35% (16,17) respectively. A similar observation can be made in cases of microvascular invasion (5 recurrences in 35 patients,14%) or poorly differentiated tumors (6 recurrences in 17 cases, 35%), where reported rates range from 20 to 47% and 13 to 63% respectively (14,17-19).

Our policy was to biopsy HCC lesions larger than 5 cm and to exclude patients with tumors that were both large and poorly differentiated, as we felt risk of tumour recurrence may be prohibitive in such a combination. This point has to be taken into consideration when interpreting data, especially of patients beyond
Milan or UCSF criteria. However, only three patients were excluded based on these criteria. As such, the impact on the overall results is probably minor as one quarter of the reported patients still demonstrated poorly differentiated tumors on pathology (usually under 5 cm in diameter). This rate is similar to those reported by most previous studies, which often do not include patients with tumors >5 cm (7,9,10,15), and remains different from studies performing biopsies in all patients and excluding all patients with poorly differentiated cancers (20).

When a recurrence of HCC did occur, patients experienced long survivals (23 +/- 26 months mean survival after recurrence), with one patient alive 6.6 years after recurrence. In contrast, previous data demonstrated early deaths after recurrence in most patients (10,18).

These observations, in keeping with case reports demonstrating HCC remission after sirolimus introduction (21), suggest that sirolimus may be at least as potent as other drugs in the prevention of HCC recurrence. It is clear however, that the possible impact of sirolimus on HCC requires confirmation by further studies.

In addition to the possibility of survival advantage, we wanted to further document the potential for adverse impact associated with sirolimus. Our study was designed as intent-to-treat and sirolimus has been stopped in some patients. However, 88 and 80% of patients were still on sirolimus at one and four years. These rates of drug discontinuation compare favourably with previously published reports on use of sirolimus in kidney transplant with ranges from 68 to
73% at one year (22,23). They are also similar to those observed with other immunosuppression protocols (24).

Most discontinuations were related to side-effects.

High rates of anemia, leucopenia and dyslipidemia were associated with sirolimus. These observations were found in several randomized studies (22-24) and identify these potential problems as requiring specific follow-up and management. Dyslipidemia appears to be less frequent when sirolimus is combined with tacrolimus as compared to cyclosporine (25). Mouth ulcers occurred in about one third of patients, but tended to clear with temporary dosage reduction and local care. Other previously reported side effects (26), like peripheral edema, joint pain and pleural effusion, were not found in the present study.

Creatinine levels demonstrated a trend to increase, especially within the first year after transplant. This suggests that the sirolimus/low-dose tacrolimus combination is associated with some degree of nephrotoxicity. Seventeen percent of patients required de novo insulin within the first year after transplant, also reflecting some diabetogenicity of the chosen immunosuppression.

Sirolimus has demonstrated some evidence of beneficial effect on HCV. Two previously reported patients cleared HCV RNA when immunosuppression has been converted to sirolimus (27). In the present report, 53% of patients had a biopsy-proven HCV recurrence, which is similar to previous series (28). While our study was not designed to specifically investigate HCV, no obvious major impact of sirolimus (positive or negative) could be identified.
Rates of incisional hernias were high, appearing in one third of the patients (they were higher than in patients with sirolimus-free immunosuppression- data not shown). While the University of Colorado did not find increased incidence of wound healing problems related to sirolimus (29), we identified, in keeping with others (7,24,30), this problem as one requiring specific attention. Abdominal wall closure should be carefully performed with high suture versus incision length ratio (at least 4:1), without tension and possibly using permanent sutures (31). In high risk patients (obese with major ascites), delay in the introduction of sirolimus should be considered in balance with potential anti-tumor impact. Finally, one should perform the bi-subcostal incision as high as possible, and avoid the subsequent vertical median split where exposure allows, this component having been reported to increases the risk of hernia (32).

The present study demonstrates that the use of sirolimus based immunosuppression post liver transplantation is safe. Common side effects included anemia, leucopenia, dyslipidemia and mouth ulcers, none of which are life threatening, and most relatively easily controlled. Because of the high rate of wound complications, select high risk patients may benefit from specific attention to technique of wound closure and/or delayed introduction of sirolimus. Overall, satisfactory outcomes can be achieved in liver transplantation using sirolimus-based immunosuppression, even in selected patients with HCC beyond Milan criteria, where tumor-free survivals were 82 and 75% at one and four years. The
potential benefit of sirolimus in patients with HCC requires confirmation by a randomized study, which may be guided by the present pilot experience.
References


function, and protocol compliance at 1 years. Transplantation 2004; 77[2]: 252.


7. Sirolimus-based immunosuppression: a registry-based study

A version of this chapter has been published in Hepatology 2010;51(4):1237-43

by Christian Toso, Shaheed Merani, David L. Bigam, A.M. James Shapiro,
Norman M. Kneteman
Abstract

Liver transplantation is an important treatment option for selected patients with nonresectable hepatocellular carcinoma (HCC). Several reports have suggested a lower risk of posttransplant tumor recurrence with the use of sirolimus and a higher one with calcineurin inhibitors, but the selection of an ideal immunosuppression protocol is still a matter of debate. The aim of this study was to define the immunosuppression associated with the best survival after liver transplantation for HCC. It was based on the Scientific Registry of Transplant Recipients and included 2,491 adult recipients of isolated liver transplantation for HCC and 12,167 for non-HCC diagnoses between March 2002 and March 2009. All patients remained on stable maintenance immunosuppression protocols for at least 6 months posttransplant. In a multivariate analysis, only anti-CD25 antibody induction and sirolimus-based maintenance therapy were associated with improved survivals after transplantation for HCC (hazard ratio [HR] 0.64, 95% confidence interval [CI]: 0.45-0.9, P ≤0.01; HR 0.53, 95% CI: 0.31-0.92, P ≤0.05, respectively). The other studied drugs, including calcineurin inhibitors, did not demonstrate a significant impact. In an effort to understand whether the observed effects were due to a direct impact of the drug on tumor or more on liver transplant in general, we conducted a similar analysis on non-HCC patients. Although anti-CD25 induction was again associated with a trend toward improved survival, sirolimus showed a trend toward lower rates of survival in non-HCC recipients, confirming the specificity of its beneficial impact to cancer patients.
Conclusion: According to these data, sirolimus-based immunosuppression has unique posttransplant effects on HCC patients that lead to improved survival.
Introduction

Liver transplantation is the treatment of choice for selected patients with non-resectable hepatocellular carcinoma (HCC). While the surgical procedure is well established, the definition of the most appropriate immunosuppression combination, allowing decreased risk of tumor recurrence and improved survival is still a matter of debate. To date, no single protocol has gained broad acceptance. In recent years, this lack of consensus has become more acute, given the increasing number of patients undergoing transplantation for HCC, currently the second commonest indication for liver transplantation in the USA, after HCV disease (www.ustransplant.org/annual_reports). We can also expect the number of transplantation for HCC to further increase, with several recent studies showing that selected patients beyond Milan criteria can be safely considered for transplantation (1-5).

Until now the positive and negative effects of various immunosuppressive drugs on HCC have been demonstrated in animal data and suggested from a few retrospective single-center clinical studies. Calcineurin-inhibitors, including both tacrolimus and cyclosporine, have been associated with a dose-dependent increase in the post-transplant risk of HCC recurrence (6). Conversely, sirolimus has shown anti-cancer properties in in vitro and animal models, both alone or in combination with doxorubicin or sorafenib (7-12). Sirolimus can prevent angiogenesis by interfering with VEGF-mediated pathways in endothelial cells, thus limiting the growth of tumors (7), and also impacts on established tumors, by inducing extensive microthrombi and so inhibiting tumor growth (9, 13). While
these animal data are clear, clinical studies are less convincing. We have demonstrated good outcomes with the use of sirolimus in a non-controlled trial, and more recently the groups of the University of Colorado in Denver and of Fudan University in Shanghai demonstrated better survivals in patients on sirolimus compared to control liver recipients (4, 14-16). While all show similar trends, these retrospective studies included limited numbers of patients, and possible confounding variables could not be taken into account due to the limited sample size.

The present study is based on a large registry transplant population and evaluates the impact of immunosuppression on survival, in an attempt to define the best post-transplant treatment combination for HCC patients.
Patients and Methods

This study analyzed data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donors, wait-listed candidates, and transplant recipients in the United States of America (US), submitted by the members of the Organ Procurement and Transplantation Network (OPTN), and has been described elsewhere (17). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors. The study has been reviewed and approved by the Health Research Ethics Board at the University of Alberta. The study population included all adult (≥16 years) patients, who received an isolated liver transplantation from March 2002 to March 2009. In order to ensure that all subjects had a significant exposure to the drugs, only individuals kept on the same maintenance immunosuppression protocol for at least 6 months post-transplant (or until death) were further selected. Overall, 25201 out of 39859 patients receiving a liver transplant during the study period were excluded.

The unique outcome variable of the study was patient survival. The occurrence and the date of death were obtained from data reported to the SRTR by the transplanting centers and were completed by data from the US Social Security Administration and from the OPTN. All deaths were taken into account in the analysis, whether they were associated to HCC or not. Of note, the SRTR data were not of sufficient granularity nor previously validated to allow the use of variables such as cancer recurrence or cancer-associated death. As a
consequence, some patients may have been alive with an HCC recurrence and were not considered as an event in the survival analyses.

A first analysis was conducted on patients transplanted for HCC only. Subjects with cholangio-carcinoma, hepatoblastoma, hemangio-endothelioma and benign liver tumors were excluded. We performed a univariate analysis, using the Kaplan Meier technique and comparing groups with log-rank tests. The impact of immunosuppression was analysed, comparing patients put on a specific drug prior to the original post-transplant discharge and kept on the same drug for at least 6 months, to those who have not been put on that specific drug for at least 6 months post-transplant. The following variables were used: tacrolimus (Prograf), cyclosporin (Sandimmune, Neoral and generics), sirolimus (Rapamune), mycophenolate mofetil (Cellcept), steroids (methylprednisolone, Solumedrol and oral prednisone, excluding patients treated with steroids for rejection episode), and induction therapy with an anti-CD25 antibody (daclizumab, Zenapax and basiliximab, Simulect) or with Thymoglobulin.

We further conducted a stepwise multivariate Cox regression analysis. The previously described immunosuppression variables were all entered in this analysis and results were corrected for the following covariates: Model for End-Stage Liver Disease (MELD) score, year of transplant, age at transplant, primary underlying liver disease, Total Tumor Volume (TTV), alpha-fetoprotein (AFP) and pre-transplant tumor treatment (yes vs. no). TTV was calculated as previously reported, by adding the volume of each HCC \( ((4/3)\pi r^3) \) based on the maximum radiological radius of each tumor (5, 18, 19). Of note, only TTV and AFP were
used as HCC factors, as they have been previously reported to be the main variables impacting on patient survival (5, 18). Data obtained on the date closest to transplant were used.

In an effort to understand whether the observed results were due to specific impacts of the drugs on HCC or more generally on liver transplantation overall, we further conducted the same univariate and multivariate analyses independently on patients transplanted during the same time period for non-HCC diagnoses. Similar variables and covariates were used, excluding those directly applicable to HCC patients: TTV, AFP and pre-transplant tumor treatment.

The study design, which only allowed the inclusion of patients on stable immunosuppression for at least 6 months post-transplant, resulted in data on immunosuppression being available for all patients. Covariates were missing in less than 35% (the most frequently missing was AFP) in the HCC group and were replaced by the mean. Covariates were complete in the non-HCC group. Other statistical tests included the use of Student-t and Chi-square tests to compare the demographic variables between groups. Results were provided as mean ± standard deviation. Standard alpha level of 0.05 indicated statistical significance. Analyses were conducted using SPSS 15.0 (SPSS, Chicago, IL).
**Results**

During the study period, 2,491 adult patients received an isolated liver transplant for HCC and 12,167 for non-HCC diagnoses (Table 7.1). All analyzed patients remained on the same maintenance immunosuppressive drugs for at least 6 months post-transplant. HCC patients included more males (female/male ratio: 1/3.9 vs. 1/1.8, p≤0.001) and were older (56 ±8 vs. 51 ±11 years on average, p≤0.001). The incidence of HCV- and HBV-induced liver disease was also higher among HCC patients (p≤0.001). Finally, calculated MELD scores, not adjusted for tumor exception points, were lower in the HCC group (14 ±6 vs. 21 ±8, p≤0.001).

As the SRTR registry is based in the US, where HCC patient selection is performed according to Milan criteria (1), only 0.2% of HCC subjects had a TTV higher than 115cm$^3$. Six percent had an AFP >400 ng/ml. As a result, the included HCCs were relatively homogenous and with similar expected outcomes (5).

The use of immunosuppressive drugs was similar between HCC and non-HCC patients. An induction therapy was used in a minority of recipients (anti-CD25 antibody: 12 and 10.8%, Thymoglobulin 6.3 and 7.3%). The most frequently used maintenance therapies were tacrolimus (90.6 and 92.5%), steroids (82.9 and 85.7%) and mycophenolate mofetil (57.6 and 59.5%).
<table>
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<tr>
<th></th>
<th>HCC patients</th>
<th>non-HCC patients</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Patients (number)</td>
<td>2491</td>
<td>12167</td>
<td></td>
</tr>
<tr>
<td>Mean age (years ±SD)</td>
<td>56 ±8</td>
<td>51 ±11</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Gender (ratio)</td>
<td>female:504/</td>
<td>female:4364/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>male:1987</td>
<td>male:7803</td>
<td></td>
</tr>
<tr>
<td>Cause of liver disease (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV (±alcohol, ±HBV)</td>
<td>1343 (54)</td>
<td>4225 (35)</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Alcohol</td>
<td>227 (9)</td>
<td>1642 (13)</td>
<td>≤0.001</td>
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<td>HBV</td>
<td>199 (8)</td>
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<td>343 (3)</td>
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<td>27 (1)</td>
<td>462 (4)</td>
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<td>19 (0.5)</td>
<td>588 (5)</td>
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</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>13 (0.5)</td>
<td>857 (7)</td>
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</tr>
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<td>20 (0.5)</td>
<td>65 (0.5)</td>
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</tr>
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<td>≤0.001</td>
</tr>
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<td>Metabolic disorder</td>
<td>7 (0.5)</td>
<td>75 (0.5)</td>
<td>0.04</td>
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<td>Acute liver necrosis</td>
<td>86 (3)</td>
<td>844 (7)</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Other</td>
<td>433 (17)</td>
<td>1567 (13)</td>
<td>≤0.001</td>
</tr>
<tr>
<td>MELD score at transplantation</td>
<td>14 ±6</td>
<td>21 ±9</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Mean Total Tumor Volume (cm³±SD)</td>
<td>17 ±39</td>
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<td></td>
</tr>
<tr>
<td>Total Tumor Volume &gt;115 cm³ (%)</td>
<td>5 (0.2)</td>
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</tr>
<tr>
<td>Mean serum alpha fetoprotein level (ng/ml ±SD)</td>
<td>291 ±1384</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Serum alpha fetoprotein level &gt;400 ng/ml (%)</td>
<td>157 (6)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus infection, HBV: hepatitis B virus infection, NA: non applicable
MELD: Model for End-Stage Liver Disease

Table 7.1: Patient and tumor characteristics
We first performed a univariate analysis based on the HCC group only. Patients receiving induction with anti-CD25 antibodies and those treated with a sirolimus-based maintenance protocol demonstrated significantly higher survivals that reached 6% and 14.4% advantages by 5 years (p ≤0.01 and p ≤0.05 respectively, Table 7.2 and Figure 7.1). On multivariate analysis, corrected for MELD score, year of transplant, age at transplant, primary underlying liver disease, TTV, AFP and pre-transplant tumor treatment, both anti-CD25 antibodies and sirolimus remained significant predictors of patient survival (Hazard Ratio 0.64, 95%CI 0.45-0.9, p ≤0.01; HR 0.53, 95%CI 0.31-0.92, p ≤0.05).
<table>
<thead>
<tr>
<th></th>
<th>HCC patients</th>
<th>Non-HCC patients</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>3-year patient survival (%)</td>
<td>5-year patient survival (%)</td>
<td>p*</td>
<td>3-year patient survival (%)</td>
<td>5-year patient survival (%)</td>
<td>p*</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>on (n=2256)</td>
<td>79.9</td>
<td>69.6</td>
<td>0.87</td>
<td>on (n=11259)</td>
<td>87.3</td>
<td>79</td>
<td>≤0.001</td>
</tr>
<tr>
<td>off (n=235)</td>
<td>77.5</td>
<td>68.2</td>
<td></td>
<td>off (n=908)</td>
<td>81.8</td>
<td>72.9</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on (n=207)</td>
<td>76.6</td>
<td>69.2</td>
<td>0.67</td>
<td>on (n=735)</td>
<td>81.8</td>
<td>72.9</td>
<td>≤0.001</td>
</tr>
<tr>
<td>off (n=2284)</td>
<td>79.9</td>
<td>69.4</td>
<td></td>
<td>off (n=11432)</td>
<td>87.2</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Sirolimus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on (n=109)</td>
<td>85.6</td>
<td>83.1</td>
<td>≤0.05</td>
<td>on (n=430)</td>
<td>84.2</td>
<td>74</td>
<td>0.14</td>
</tr>
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<td>off (n=2382)</td>
<td>79.2</td>
<td>68.7</td>
<td></td>
<td>off (n=11737)</td>
<td>87</td>
<td>78.7</td>
<td></td>
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<tr>
<td>Mycophenolate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>moftil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on (n=1434)</td>
<td>78.3</td>
<td>68.5</td>
<td>0.32</td>
<td>on (n=7244)</td>
<td>87</td>
<td>78.8</td>
<td>0.62</td>
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<tr>
<td>off (n=1057)</td>
<td>81</td>
<td>70</td>
<td></td>
<td>off (n=4923)</td>
<td>86.8</td>
<td>78.2</td>
<td></td>
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<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on (n=2066)</td>
<td>79</td>
<td>68.5</td>
<td>0.07</td>
<td>on (n=10429)</td>
<td>86.8</td>
<td>78.1</td>
<td>0.11</td>
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<tr>
<td>off (n=425)</td>
<td>82.7</td>
<td>73.8</td>
<td></td>
<td>off (n=1738)</td>
<td>87.5</td>
<td>80.6</td>
<td></td>
</tr>
<tr>
<td>Induction: anti-CD25 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on (n=299)</td>
<td>87.9</td>
<td>74</td>
<td>≤0.01</td>
<td>on (n=1320)</td>
<td>88</td>
<td>78.7</td>
<td>0.6</td>
</tr>
<tr>
<td>off (n=2192)</td>
<td>78.5</td>
<td>68</td>
<td></td>
<td>off (n=10847)</td>
<td>86.7</td>
<td>78.5</td>
<td></td>
</tr>
<tr>
<td>Induction: thymoglobulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on (n=158)</td>
<td>75.6</td>
<td>66.3</td>
<td>0.44</td>
<td>on (n=894)</td>
<td>88.3</td>
<td>79.9</td>
<td>0.5</td>
</tr>
<tr>
<td>off (n=2333)</td>
<td>79.8</td>
<td>69.6</td>
<td></td>
<td>off (n=11273)</td>
<td>86.8</td>
<td>78.4</td>
<td></td>
</tr>
</tbody>
</table>

* Log-rank tests

Table 7.2: Univariate analysis of factors impacting on patient survival
Figure 7.1: Kaplan-Meier analysis of survival after liver transplantation for HCC.
Both sirolimus (A, $p \leq 0.05$) and anti-CD25 antibody induction (B, $p \leq 0.01$) demonstrated significantly improved survivals.
Of note, the protective effect of sirolimus did not appear to be linked to a selection bias, as patients on sirolimus demonstrated higher MELD scores than those sirolimus-free (15 ±7 vs. 14 ±1, p=0.02). In addition, the other studied characteristics were either similar between both groups, or are without known impact on HCC-free post-transplant survival (Table 7.3).

<table>
<thead>
<tr>
<th>HCC patients on sirolimus</th>
<th>HCC patients sirolimus-free</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (number)</td>
<td>109</td>
<td>2382</td>
</tr>
<tr>
<td>Mean age (years ±SD)</td>
<td>56 ±6</td>
<td>56 ±2</td>
</tr>
<tr>
<td>Gender (ratio)</td>
<td>female:32/ male:77</td>
<td>female:472/ male:1910</td>
</tr>
<tr>
<td>Cause of liver disease (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV (±alcohol, ±HBV)</td>
<td>52 (57)</td>
<td>1291 (54)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>7 (6)</td>
<td>220 (9)</td>
</tr>
<tr>
<td>HBV</td>
<td>4 (4)</td>
<td>195 (8)</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>4 (4)</td>
<td>86 (3.5)</td>
</tr>
<tr>
<td>NASH</td>
<td>1 (1)</td>
<td>46 (2)</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>2 (2)</td>
<td>25 (1)</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>1 (1)</td>
<td>18 (0.5)</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>0</td>
<td>13 (0.5)</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>1 (1)</td>
<td>19 (0.5)</td>
</tr>
<tr>
<td>Alpha1-antitrypsin deficiency</td>
<td>2 (2)</td>
<td>5 (0.5)</td>
</tr>
<tr>
<td>Metabolic disorder</td>
<td>1 (1)</td>
<td>6 (0.5)</td>
</tr>
<tr>
<td>Acute liver necrosis</td>
<td>1 (1)</td>
<td>85 (4)</td>
</tr>
<tr>
<td>Other</td>
<td>33 (30)</td>
<td>373 (16)</td>
</tr>
<tr>
<td>MELD score at transplantation (±SD)</td>
<td>15 ±7</td>
<td>14 ±1</td>
</tr>
<tr>
<td>Mean Total Tumor Volume (cm³ ±SD)</td>
<td>19 ±2.3</td>
<td>17 ±1.1</td>
</tr>
<tr>
<td>Total Tumor Volume &gt;115 cm³ (%)</td>
<td>0</td>
<td>5 (0.2)</td>
</tr>
<tr>
<td>Mean serum alpha fetoprotein level (ng/ml ±SD)</td>
<td>147 ±72</td>
<td>297 ±36</td>
</tr>
<tr>
<td>Serum alpha fetoprotein level &gt;400 ng/ml (%)</td>
<td>3 (1)</td>
<td>154 (6)</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus infection, HBV: hepatitis B virus infection
MELD: Model for End-Stage Liver Disease

Table 7.3: Characteristics of HCC patients on or off sirolimus
In an effort to understand whether the positive impact of these drugs on survival was linked to a specific effect on HCC or to a general effect on liver transplantation, we conducted a similar analysis on the non-HCC group. Based on this analysis, a drug having a positive effect on the HCC group and a negative effect on the non-HCC group should be assumed to have a mode of action with beneficial impact mainly targeting HCC. This could be the case for a drug holding anti-cancer properties. Conversely, a drug associated with improved survivals on both HCC and non-HCC patients should be assumed to act on the outcome of liver transplantation in general.

When looking at the non-HCC group and performing a univariate analysis, tacrolimus-based therapy was associated with improved survivals, while cyclosporine-based treatment was linked to decreased survivals (Table 7.2). On multivariate analysis, only the use of cyclosporine-based maintenance protocols remained associated with decreased outcomes (HR 1.3, 95%CI 1-1.7, p ≤0.05).

In order to better understand the effects of the various studied drugs on patient survival, we have plotted the HR (± 95% confidence intervals) found in the HCC and non-HCC groups (Figure 7.2). Of the significant variables in the multivariate analyses, both anti-CD25 antibodies and cyclosporine demonstrated trends in similar directions in both groups, suggesting that their effect was primarily directed towards liver transplant in general (and not specifically towards HCC). Conversely, sirolimus-based immunosuppression had a trend toward a protective effect in the HCC group and a negative impact in the non-HCC group, thus suggesting that the significant effect of this drug was primarily directed towards
HCC. Of all the studied protocols, sirolimus-based immunosuppression was the only one showing such a pattern.

As described earlier, the SRTR does not include data on HCC recurrence. In order to approximate this data, we have performed an assessment of patients dying of malignancy, and have shown that twice as many patients not on sirolimus died from cancer (HCC or other) compared to those on sirolimus (11% vs 5% respectively at 5 years). While this observation did not reach significance (p=0.15, log-rank), possibly due to the low report rate of this variable, the observed trend further supports the message of the study.
Figure 7.2: Hazard Ratios (± 95% confidence intervals) to compare the risk of mortality after liver transplantation using various immunosuppression protocols. Results were corrected for MELD, year of transplant, primary liver disease (non HCC), age at transplant, and when applicable, Total Tumor Volume, alpha-fetoprotein and pre-transplant HCC treatment. * corresponds to significant variables.
Discussion

According to the present SRTR registry data, the use of sirolimus-based immunosuppression protocols has unique beneficial post-transplant effects on HCC patients, leading to significantly improved survival. Our analysis of the SRTR data set also suggests that anti-CD25 antibody induction is associated with a longer post-transplant life expectancy (significant for HCC and with a trend for non-HCC).

While the anti-cancer effect of sirolimus has been suggested by previous animal and single-center studies (7-12), the present data is the first to be adequately powered. It highlights a key potential role for this agent in patients undergoing liver transplantation for HCC. It should also encourage both physicians and regulatory agencies to consider removal of the “black box” linked to the use of sirolimus in liver transplantation (www.fda.gov). This warning was put forward by the FDA in 2002, based on phase II trials showing a significantly increased incidence of early post-transplant hepatic artery thrombosis and infection-associate deaths (www.fda.gov). Sirolimus has now passed the test of time in several centers, all reporting no increase in the incidence of these complications (14, 20). Like any potent immunosuppressive drug, sirolimus is linked to a potential for development of numerous side-effects, including dyslipidemia, peripheral edema, anemia, leukopenia, delayed wound healing and a substantially increased risk of incisional hernia (14, 21, 22). In general however, we believe that these side-effects are relatively minor and easy to manage, and
that the data revealed by the present study justify a broader use of protocols including sirolimus after liver transplantation for patients with HCC.

It should be clearly emphasized that this study was not designed to look at the effect of specific drugs, but rather reports on protocols containing specific drugs. We had no access to drug doses or trough levels. As such, while it sounds logical that the improved survival associated with sirolimus-containing protocols is the result of its anti-cancer effects, we cannot rule out that lower doses of CNIs were used in these patients, perhaps reinforcing the effect of sirolimus (6).

Of all protocols, sirolimus-based immunosuppresion was the only one associated with an improved post-transplantation survival specific to HCC patients (and not to non-HCC patients), further reinforcing the clinical evidence of its anti-cancer properties. The use of anti-CD25 antibodies demonstrated similar trends to improved survival in both HCC and non-HCC patients. These observations, together with previous reports combining anti-CD25 antibody induction and delayed introduction of CNIs, speak in favor of the use of this drug after liver transplantation in general (23). Finally, the present data also supports the use of tacrolimus-based rather than cyclosporine-based maintenance immunosuppression after liver transplant.

The registry nature of the study is linked to several limitations. We did not have access to data on HCC recurrence, which would have been useful to better define the anti-cancer impact of the drugs. However, due to the lack of access to effective treatment, most patients with HCC recurrence post-transplant are expected to die from the disease, making the rate of survival a reasonable
marker. In addition, most deaths occurring during the first 5 years after transplantation for HCC are related to tumor recurrence (and not other causes like HCV recurrence) (14). In an effort to provide better understanding of the anti-HCC effect of the drugs, we studied both HCC and non-HCC groups separately and subsequently compared them, thus providing a good reading of the effect of sirolimus-based therapies on HCC.

In order to further decrease the risk of bias, the multivariate analyses have been corrected for multiple variables that could potentially impact on survival. To illustrate, the opposite results linked to sirolimus between the HCC and non-HCC groups could have been linked to differences in the indications for the use of the drug. The use of sirolimus was linked to the presence of HCC in one group, and may have been used to spare the use of CNIs in the other group, especially in patients with renal dysfunction. While the risk of bias was decreased by the integration of pre-transplant MELD in the analysis, we could not completely rule out differences in post-transplant kidney function between groups.

According to the present study, sirolimus-based immunosuppression is associated to improved patient survival after liver transplantation for HCC. Anti-CD25 antibody induction demonstrates a similar effect in patients transplanted for HCC and non-HCC diagnoses. We believe that these data will help in the transplant management of HCC patients, integrating a balanced selection of candidates with expected good outcomes and a post-transplant adjuvant
treatment including appropriate and effective immunosuppression with anti-cancer properties.

**Acknowledgements**

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References


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20. Dunkelberg JC, Trotter JF, Wachs M, Bak T, Kugelmas M, Steinberg T, et al. Sirolimus as primary immunosuppression in liver transplantation is not
associated with hepatic artery or wound complications. 9 ed. 2003. 463-468.


8. Sirolimus-based immunosuppression after living donor liver transplantation

A version of this chapter has been published in Clinical Transplantation 2010, in press

by Christian Toso, Seema Patel, Sonal Asthana, Toshiyasu Kawahara, Safwat Girgis, Norman N Kneteman, AM James Shapiro, David L Bigam
Abstract

There is a lack of data on the use of sirolimus after partial liver transplantation, especially regarding its impact on post-transplant regeneration.

We reviewed adult living donor transplantations, with de novo sirolimus (n=7) and without sirolimus (n=21). Liver biopsies were stained for KI-67, a proliferation marker. Controls included specimens with normal liver parenchyma (n=13). Both groups had similar demographics, graft and patient survival and complication rates. During the first six wk and over the whole first year post-transplant, the use of sirolimus was associated with lower levels of hepatocyte proliferation compared to sirolimus-free patients, (overall, 0.3 [0-7.2] vs. 3 [0-49] KI-67 positive hepatocytes per high power field, p ≤0.05). The levels observed in the sirolimus group were similar to those seen in non-transplanted control patients with normal parenchyma (0.2 [0-1.3], p=NS). Post-transplant hepatocyte proliferation correlated with the serum levels of sirolimus (p ≤0.05), but not with those of tacrolimus or with the dose of mycophenolate mofetil (p=0.9 and 0.3, respectively).

These data suggest that sirolimus is associated with decreased post-transplant hepatocyte proliferation. The clinical significance of this observation remains to be fully determined.
**Introduction**

Sirolimus represents an important immunosuppressive option after liver transplantation, especially in patients with calcineurin inhibitor-induced neurotoxicity, renal failure (1), and possibly also in cases of hepatocellular carcinoma (2,3).

The use of de novo sirolimus however, has remained limited (3-7), mainly because of a “Black Box” warning from the US Food and Drug Administration. This followed two multicenter phase II/III trials (8,9), suggesting that sirolimus was associated with a trend towards increased rates of hepatic artery thrombosis within the first 3 weeks post-transplant. Although the trend has not been confirmed by subsequent studies (3-5,7,10), the warning remains.

Living donor transplantation bears further specific challenges, including an increased risk of vascular complications as compared to whole organ transplantation. As such, and because of the observations described earlier, the use of de novo sirolimus has remained anecdotal in this patient group (7). In addition, liver transplantation from living donors is performed with partial grafts, requiring post-transplant regeneration. This post-transplant growth may be prevented by sirolimus, as *in vitro* and animal reports have demonstrated an anti-proliferative effect of this drug, as part of its anti-tumour propriety (11,12).

In order to assess the impact of de novo sirolimus after living donor transplantation, we looked at clinical outcomes and at rates of post-transplant hepatocyte proliferation.
Patients and Methods

Inclusion criteria

This study was conducted retrospectively to determine the impact of sirolimus on clinical outcomes and hepatocyte proliferation after living donor transplantation in adults. It has been reviewed and approved by the Institutional Review Board Committee at the University of Alberta.

All living donor liver transplantations performed in adult (over 18 years) recipients from June 2001 to March 2007 were included in the study. Patients were divided into two groups, those who were put on de novo sirolimus and those who were not. The patients on de novo sirolimus were transplanted prior to 2003, except one transplanted later for hepatocellular carcinoma (we currently avoid the use of sirolimus in case of live donor transplantation, because of the absence of strong data defining the risk profile and the “black box” warning from the FDA).

Outcomes assessment

Data was collected prospectively in an electronic database (OTTR, Hickman-Kenyon Systems, Omaha, NE) and analyzed retrospectively.

Living donor liver transplants were performed utilizing both related and unrelated donors. A standardized pre-donation assessment was performed in all cases. This pre-donation assessment included ultrasound, CT-scanning with volumetry, magnetic resonance cholangiography and clinical assessments by a surgeon, hepatologist, nutritionist, social worker and an independent physician outside the Program. Graft to recipient weight ratio (GRWR) was based on CT volumetry.
Primary graft function was assessed by the peak ALT and AST (defined as the highest value recorded on daily blood works performed until day 5) and the INR on day 2, as described previously (13). Primary non-function was defined as the need for early re-transplantation in the absence of surgical (biliary or vascular) complication. Complications were assessed for the first year after transplantation only.

*Histological proliferation assessment*

All liver biopsies performed during the first year post-transplant were reviewed and included in the study. These had been performed according to clinical need, mainly due to elevated liver function tests. One previously non-stained slide was used at each time point for KI-67 staining. This staining is specific for cell proliferation, as the KI-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent in resting cells (G0)(14). For comparison, a negative control group (without regeneration stimulus) included 13 specimens of normal liver parenchyma. These were obtained at the time of liver resection for colorectal metastases (n=8), neuroendocrine metastases (n=3), or various benign focal diseases (n=2). The median age of the control group was 47 years [23-66] and demonstrated normal or close to normal liver function tests; bilirubin: 11 [6-22] μmol/l, alkaline phosphatase: 81 [41-112] U/l and ALT: 23 [9-47] U/l. An esophageal specimen was used as the positive control. All slides were processed at the same time.
The KI-67 staining was performed according to a standard clinical protocol at the Department of Pathology, University of Alberta hospital. After antigen retrieval, slides were incubated with mouse anti-human anti-KI-67 antibody (clone MIB-1, DAKO, Mississauga, Ontario). A biotinylated anti-mouse secondary antibody was further applied, and streptavidin-horseradish peroxidase and diaminobenzidine were used to visualize bound antibodies.

KI-67 stained slides were assessed blindly. For each slide, all KI-67 positive hepatocyte nuclei were counted in ten high power magnification (400x) fields and the mean was reported. The counts were performed on a NIKON Eclipse E400 microscope; the high power magnification field area measured 0.83 mm$^2$. The rates of high, medium and low intensity staining were also assessed.

**Statistical analyses**

Results were reported as median [minimum-maximum values]. Categorical variables were studied using the Fisher test. Continuous variables were assessed by non-parametric tests, including Mann-Whitney and Kruskall-Wallis tests. KI-67 results were compared between groups, using data collected during the first six weeks post-transplant (when most of the regeneration occurs) or during the first year post-transplant. Survival was analyzed by the Kaplan-Meier method and differences between groups were further tested by the log-rank test. Relations between continuous variables were studied using Spearman rank correlation test. Such correlations were performed between the number of KI-67 positive hepatocytes per high magnification field (assessed during the first six
weeks) and the area under the curve (AUC) of the serum levels of sirolimus and tacrolimus or the dose of mycophenolate mofetil (MMF). These AUCs were calculated using the trapezoidal rule and computing serum levels at 3, 7, 14 and 42 days or up to the time of biopsy (15). They provided a measure of the exposure to the drug until biopsy, as previously described (15). P values less than 0.05 were considered significant. Calculations were performed using SPSS 15.0 software (Chicago, IL).
Results

Demographics

During the study period, 361 adult patients received a liver transplant, of which 28 were performed from a living donor. Seven of these 28 were placed on sirolimus within the first week post-transplant (SRL). These were compared to the other 21 patients not receiving sirolimus (non-SRL). Both groups had similar characteristics (recipient age, gender, type of graft, donor age, GRWR, cause of liver disease and MELD; Table 8.1), however the patients with the longest follow-ups (73 vs. 37 months, p≤0.01) and those diagnosed with primary sclerosing cholangitis were more often on sirolimus (Table 8.1).

Immunosuppression

Induction therapy with daclizumab (Zenapax, Hoffman-La-Roche, Mississauga, Ontario, Canada) was used in all patients, except three (one in the SRL and two in the non-SRL groups). All patients in the SRL group were on a combination of tacrolimus (Prograf, Astellas, Markham, Ontario, Canada, trough levels 3 to 6 μg/l) and sirolimus (Rapamune, Wyeth Research Laval, Quebec, Canada, trough levels 10 to 18 μg/l). Generally, sirolimus was started from the first day post-transplant, except in one patient who was started on day 5. Patients in the non-SRL group were put on tacrolimus (trough levels 7 to 15 μg/l), together with MMF (n=19; Cellcept, Hoffman-La-Roche, Mississauga, Ontario, Canada) and/or short-term steroids (n=12).
<table>
<thead>
<tr>
<th></th>
<th>SRL (%)</th>
<th>noSRL (%)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>51 [23-66]</td>
<td>54 [26-69]</td>
<td>0.8</td>
</tr>
<tr>
<td>Gender</td>
<td>female:3/ male:4</td>
<td>female:9/ male:12</td>
<td>1</td>
</tr>
<tr>
<td>Right/Left lobe graft</td>
<td>7/0</td>
<td>16/5</td>
<td>0.3</td>
</tr>
<tr>
<td>Graft to recipient weight ratio (%)</td>
<td>1.2 [1-2]</td>
<td>1.4 [0.9-1.9]</td>
<td>0.4</td>
</tr>
<tr>
<td>Median donor age (years)</td>
<td>38 [22-54]</td>
<td>35 [18-54]</td>
<td>0.8</td>
</tr>
<tr>
<td>Cause of liver disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV (±alcohol, ±HBV)</td>
<td>1 (14)</td>
<td>6 (29)</td>
<td>0.6</td>
</tr>
<tr>
<td>HBV</td>
<td>0</td>
<td>1 (5)</td>
<td>1</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0</td>
<td>4 (19)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>2 (29)</td>
<td>1 (5)</td>
<td>0.1</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>0</td>
<td>5 (23)</td>
<td>0.3</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>4 (57)</td>
<td>3 (14)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemangio-endothelioma</td>
<td>0</td>
<td>1 (5)</td>
<td>1</td>
</tr>
<tr>
<td>Raw MELD score</td>
<td>13 [5-23]</td>
<td>13 [0-42]</td>
<td>0.8</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus infection  
HBV: hepatitis B virus infection

Table 8.1: Demographics and patient characteristics
Graft Function, Clinical Outcome and Complications

There were no cases of primary non-function. Early graft function was similar in patients in the SRL group compared to those in the non-SRL group, as assessed by the INR on day 2 (1.9 [1.3-2.2] vs. 1.5 [1.2-3.7], p=0.6) and by the peak AST (335 [276-1876] U/l vs. 359 [180-2609] U/l, p=0.9) and ALT (288 [195-1539] U/l vs. 305 [49-2299] U/l, p=0.9). The same observation was made when normalizing results for graft volume (peak AST/volume, 0.4 [0.2-2.8] vs. 0.41 [0.15-2.8] U/(l*ml), p=0.9 and peak ALT/volume, 0.3 [0.1-2.3] vs. 0.4 [0.1-2.5] U/(l*ml), p=0.9).

Patient and graft survivals were similar between the two groups. One year patient survival was 100 and 90% (p=0.6), and graft survival 86 and 81% (p=0.9) in the SRL and non-SRL groups. The rate of rejection was three times higher in the SRL group compared to the non-SRL (57 vs. 19%, p=0.1, Table 8.2). Surgical (biliary, hepatic artery and portal vein thrombosis) and infectious complications were similar (Table 8.2). While increased rates of post-transplant wound infections have previously been associated to the use of sirolimus (2), this was not the case in the present study, possibly because of the limited sample size (Table 8.2).
<table>
<thead>
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<th></th>
<th>SRL (%)</th>
<th>noSRL (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejections</td>
<td>4 (57)</td>
<td>4 (19)</td>
<td>0.1</td>
</tr>
<tr>
<td>Surgical complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary complications</td>
<td>3 (43)</td>
<td>11 (52)</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic artery thrombosis</td>
<td>0</td>
<td>3 (14)</td>
<td>0.6</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>0</td>
<td>1 (5)</td>
<td>1</td>
</tr>
<tr>
<td>Infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial infections*</td>
<td>9 (128)</td>
<td>30 (142)</td>
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</tr>
<tr>
<td>Wound infection</td>
<td>1 (14)</td>
<td>3 (14)</td>
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</tr>
<tr>
<td>CMV</td>
<td>0</td>
<td>2 (9)</td>
<td>1</td>
</tr>
<tr>
<td>HSV I</td>
<td>1 (14)</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Fungal infections</td>
<td>0</td>
<td>4 (19)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* included all kinds of infections, but excluded wound infections

Table 8.2: Complications
**In Vivo Hepatocyte Proliferation**

In total, 10 biopsies were assessed in the SRL (with all patients still on sirolimus at the time of biopsy) and 22 in the non-SRL groups. In both groups, the levels of KI-67 were higher during the first six weeks post-transplant, reflecting enhanced hepatocyte proliferation after partial liver transplantation. Recipients on sirolimus demonstrated significantly lower levels of KI-67 positivity than those on sirolimus-free immunosuppression (0.3 [0-7.2] vs. 3 [0-49], Figure 8.1). The difference reached statistical significance (Mann-Whitney test) when considering biopsies performed during the first six weeks or during the first year post-transplant (p≤0.05 in both cases). Patients on sirolimus demonstrated similar proliferation levels to negative controls without proliferation stimulus (0.2 [0-1.3], p=0.5). Distribution between high, medium and low intensity hepatocyte staining was similar in all three groups (overall, high: 9%, medium: 76% and low: 15%). The rate of proliferation correlated negatively with the exposure to sirolimus (Figure 8.2, p≤0.05), but not to tacrolimus or mycophenolate mofetil (p=0.9 and 0.3 respectively). Of note, similar correlations were achieved when using the drug level AUCs or the levels on the day of biopsy.

There was no relation between rejection and hepatocyte proliferation (p=0.8). The KI-67 activity ranged from 0.4 to 5, in the case of rejection in the SRL group, and from 0.2 to 4.3 in the non-SRL group. In addition, there was no statistically significant relation between the level of KI-67 positive hepatocytes and the type of graft (right vs. left lobe, p=0.06), the occurrence of a biliary complication or GRWR (p=0.6 and 0.2 respectively).
Figure 8.1: In vivo post-transplant hepatocyte proliferation assessment by KI-67 staining. (A) Patients on de novo sirolimus (sirolimus) demonstrated less KI-67 positive hepatocytes per high power field (400x) than patients not on sirolimus (No sirolimus, p ≤0.05, Mann-Whitney). (B) KI-67 staining, 9 days after transplant in a patient free of sirolimus. (C) KI-67 staining, 16 days after transplantation in a patient on sirolimus.
<table>
<thead>
<tr>
<th></th>
<th>SRL (%)</th>
<th>noSRL (%)</th>
<th>p</th>
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<tr>
<td>Rejections</td>
<td>4 (57)</td>
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<td>0.1</td>
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<tr>
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</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>0</td>
<td>1 (5)</td>
<td>1</td>
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<tr>
<td>Infections</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bacterial infections*</td>
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<td>30 (142)</td>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>HSV I</td>
<td>1 (14)</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Fungal infections</td>
<td>0</td>
<td>4 (19)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* included all kinds of infections, but excluded wound infections

**Figure 8.2:** Correlation between post-transplant hepatocyte proliferation (number of KI-67 positive hepatocyte/ high magnification field assessed during the first six weeks post-transplant) and sirolimus serum level AUC (computed utilizing levels on days 3, 7, 14 and 42 or until the day of biopsy) (p≤0.05, Spearman correlation).
Discussion

This study suggests that sirolimus inhibits hepatocyte proliferation after living donor liver transplantation.

Until now, the potential impact of sirolimus after partial liver transplantation has remained poorly defined and mainly extrapolated from animal studies. While some investigators have described a decreased rate of hepatocyte proliferation and liver regeneration associated to sirolimus (16-19), others have reported an improved survival after small-for-size liver transplantation in rats (20). In the present study, we demonstrate, for the first time in humans, that sirolimus is associated with decreased hepatocyte proliferation after partial liver transplantation. This observation is supported by the dose/response effect of sirolimus (Figure 2), which provides important evidence supporting the argument that this drug inhibits hepatocyte proliferation (higher dose leads to less proliferation). This effect appeared unique to sirolimus, as tacrolimus and MMF did not demonstrate similar patterns.

The clinical relevance of these observations still remains to be further explored. On the one hand, one can assume that a rapidly regenerating liver would have the potential to produce a higher hepatic mass and accelerate post-transplant patient recovery; but on the other hand, one can also postulate that highly proliferating hepatocytes devote most of their energy to this task and may be less metabolically active.
Although the present study included a limited number of patients, the reasonably good clinical outcomes achieved speak in favour of sirolimus. In particular, similar patient and graft survivals were observed. It should also be noted that we did not encounter any hepatic artery thrombosis in the sirolimus group (vs 14% in the non-SRL group), further challenging the FDA “black box” warning. Infectious and surgical complications were distributed similarly between the two groups. Although the rates of bacterial infections appeared high in both groups, it should be emphasize that they represent all the infections observed during the first year post-transplantation, and only half of them (49%) were seen during the first month. In addition, all types of infections were recorded (including urinary tract infection, pneumonia, cellulitis, colitis and esophagitis), and many of them were minor and easily treated. Of note, the higher rate of rejection observed with the sirolimus-based immunosuppression may represent a limitation of this protocol.

The present study bears the limitations inherent to a retrospective analysis. A longitudinal assessment of liver volumes would have been of interest, but patients were followed by ultrasound and the number of available CT-scans was insufficient for an accurate reading. While this study is retrospective and using non-protocol biopsies, factors potentially impacting post-transplant regeneration (donor age, type of graft, bilirubin, rejection, infection, biliary complication, GRWR (21)) were homogeneously distributed between the two groups, minimizing the risk of bias. Another limitation is linked to the low number of patients included in the sirolimus group. While this observation increases the risk of type II error, it makes the detection of significant differences more difficult and
underlines the magnitude of the impact of sirolimus on post-transplant regeneration.

Overall, this study suggests decreased hepatocyte proliferation after partial liver transplantation, but the clinical significance of this observation remains to be fully explored and understood. This is especially true when considering that we report reasonably good clinical outcomes associated to the use of sirolimus after live donor liver transplantation.

Acknowledgements

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References


9. Discussion and future developments
In the absence of metastasis and major vessel invasion, liver transplantation is currently the best treatment for patients with limited hepatocellular carcinoma (HCC). It is associated with post-transplant survival rates >70% at 5 years in most centers (1, 2).

At the present time, a wide range of criteria is used for the selection of transplant candidates. As shown in Chapter 4, the allowed maximum HCC diameter is between 5 and 8 cm, and the allowed tumor number varies from 3 to no restriction (Figure 4.3;(1, 3-15)). Overall, the original score described by Dr. V. Mazzaferro in Milan in 1996 is the most restrictive (1 HCC ≤5cm or ≤3 HCCs each ≤3cm), and the most liberal is the one introduced by the group of Zhejisang University School of Medicine in Hangzhou (any HCC number, with total tumor diameter ≤8cm, or grade I and II with AFP ≤400 ng/ml) (3, 11).

This large variety of scores illustrates the current controversy linked to pre-transplant patient selection. Several investigators have, demonstrated that a careful expansion of inclusion criteria can lead to the selection of more transplant candidates, without altering survival. The most studied and validated score was developed at the University of California, San Francisco (UCSF) (4, 16-19).

These observations have been recognized as a sign that Milan criteria are too restrictive and should be expanded. The debate remained on how this should be done.

As shown in Figure 9.1, the acceptance of a more liberal policy should be based on the observation that the newly recruited patients (beyond Milan, but within the new score) have stable and acceptable intent-to-treat survivals compared to
those within Milan criteria. This type of selection could be performed by expanding current Milan-based selection criteria, or by allowing downstaging.

Figure 9.1: Various pre-transplant patient selection strategies
Expanding selection criteria

The backbone of most scores is a combination of HCC size and number, with or without the addition of alpha-fetoprotein (AFP) or tumor grade. In Chapters 2 and 3, we introduced the concept of total tumor volume (TTV) for patient selection. This score was calculated as the sum of the volumes of all tumors \((\frac{4}{3}\pi r^3)\), \(r\): maximum radius of each HCC).

The TTV concept, with \(r^3\) in the formula, mimics the exponential growth of tumors (Figure 9.2) and reflects the increased aggressiveness of the largest HCCs. It is also based on the observation that HCC size has much more impact on post-transplant survival than HCC number (20-23). In addition, the occurrence of micro-satellite HCC metastasis (which can be viewed as the risk of distant metastasis) increases exponentially with tumor size, matching the increase in tumor volume (24)(Figure 9.3). Finally, TTV has been shown to predict survival after liver resection of colorectal metastasis (25).
Figure 9.2: exponential tumor growth

Figure 9.3: Distance between main HCC and micro-satellite metastasis according to main HCC size. Grey line approximates tumor volume according to size.
Adapted from (24).
The use of TTV is associated with an improved radiological accuracy. Compared to Milan or UCSF criteria, more patients are correctly classified within or beyond TTV by radiology, and when using pathology size and number as gold standard (Chapter 2). Accuracies were 91%, 75% and 69% for TTV, UCSF and Milan (p≤0.001). This improved radiological accuracy represents, in my view, a clear advantage of TTV, allowing a better and fairer assessment of patients pre-transplant. It is linked to the improved ability of radiology to identify and characterize larger nodules, and their higher weight in the TTV score (26). Due to r³, large HCCs count more than smaller ones.

Another advantage of TTV is the absence of HCC number cut-off. Many scores currently used, including Milan and UCSF, only consider patients with up to three HCCs. Again, while many studies have demonstrated the low impact of HCC number on post-transplant patient survival, it appears critical to avoid such cut-offs (14, 15).

Based on a preliminary analysis carried out on HCC patient data from the Alberta Liver Transplant Program (n=52), a TTV cut-off of 115 cm³ was chosen. This cut-off was selected with use of a ROC curve based on the risk of recurrence. This could be achieved with good diagnostic accuracy (Area Under Curve: 0.8) and leading to a sensitivity of 71%, specificity 84%, positive predictive value (PPV) 42% and negative predictive value (NPV) 95%. The same test performed on the patient population at the University of Colorado (n=82) also selected 115 cm³ as cut-off.
Subsequent validation was carried out on a population of 288 patients from the Universities of Alberta, Colorado and Toronto (Chapter 2). While more patients met qualifying criteria for transplantation with TTV (28 to 53% more than Milan and 16 to 26% more than UCSF), no deterioration of outcome was demonstrated on analysis of patients within TTV ≤115 cm$^3$, in comparison to those meeting Milan or UCSF classifications at all institutions. Patients with TTV >115 cm$^3$, experienced more recurrences and lower patient survivals (p <0.05).

These results were further validated in a large Scientific Registry of Transplant Recipients-based study, which performed an overview of 6478 adult recipients of an isolated first liver transplant (Chapter 3). From March 2002 to January 2008, less than 5% of patients were transplanted outside Milan criteria, as per UNOS rules. This said, an increasing proportion of patients outside Milan criteria and with large TTV (p≤0.001) has been transplanted over time. This observation can be viewed as an overall agreement that current Milan criteria can be expanded. Of all tested variables (tumor number, largest tumor size, Milan and University of California San Francisco UCSF criteria), only Total Tumor Volume (TTV, p≤0.05) and alpha fetoprotein (AFP, p≤0.001) could predict post-transplant patient survival. While these two parameters demonstrated independent behaviours (no patient demonstrated an increase in both values), a composite score was defined with patients with TTV >115 cm$^3$ and/or AFP >400 ng/ml being outside criteria. The combined TTV/AFP score efficiently predicted post-transplant survival
(HR=2, 95% CI=1.7-2.4, p≤0.001), and patients not meeting these criteria had a survival below 50% at three years.

The messages from these studies are (1) that Milan criteria are too restrictive and should be expanded, (2) the validation of TTV as being the best available predictor of survival in a large independent group of patients, and (3) the critical need to combine morphological (TTV) and biological (AFP) variables for an accurate patient selection. To illustrate, a patient with small HCCs within Milan criteria should not be considered for transplant if AFP is high, because of the high risk of vessel invasion and/or undiagnosed metastasis.

Like TTV/AFP, other scores have been developed recently, and the exact number of potential newly recruited patients remained unclear. The study, described in Chapter 4, assessed 270 patients diagnosed with HCC in the province of Alberta, Canada. The potential number of transplant candidates was calculated based on age (≤65 years), absence of metastases and macro-vascular invasion, and on 12 previously published, expanded selection criteria. A wide range of increase in the number of transplant candidates was observed (12-63% compared to Milan), but the increase linked to TTV/AFP score was reasonable (20%) and similar to the one observed with the UCSF score (20%) (4). This data will likely assist Centers and policy agencies in predicting the need for resources linked to an expansion of criteria.
**Downstaging prior to listing and transplantation**

As illustrated by Figure 9.1, expanding selection criteria is only one way of increasing the number of transplant candidates. The alternative option is the use of tumor downstaging prior to listing (Chapter 5). Therapies such as transarterial chemo-embolisation, transarterial radioembolisation, percutaneous ethanol injection and radio-frequency ablation can decrease the size (and overall viability) of the tumours, thus potentially increasing the proportion of patients qualifying for transplantation.

Most recent studies demonstrated that downstaging is a viable strategy in selected patients, offering similar outcomes to those of patients initially within Milan criteria (27, 28). The difficulty is to define how far beyond Milan, patients should be selected for downstaging and when they could be considered for transplantation.

While further validation is still needed, current evidence suggests that patients with solitary HCCs up to 8 cm in diameter and with up to 5 tumours (all ≤4 cm with total tumour diameter ≤12 cm) can be considered for downstaging (27, 28). These cut-offs may also be combined within the Total Tumor Volume score (250 cm$^3$, corresponding to a single 7.8 cm HCC, or any size and number of HCC combination, with total volume ≤250 cm$^3$). Patients who reach traditional Milan criteria after downstaging (inactive tumours counting as zero), with no contraindication to transplantation appearing during a waiting time of at least 3 months (ideally 6 months) have a more favourable biology, as shown by a low recurrence rate. They appear as reasonable candidates for transplantation. Of
note, these criteria should be further refined by adding a more biological variable, like AFP, as a selection marker. This is required as a missed metastasis will not be treated by local treatment, and would only be detected by a high AFP, persisting despite successful local HCC downsizing.

Overall, selected patients with HCCs beyond Milan criteria should be considered for downstaging and subsequent liver transplantation. This should be performed within reason, utilizing conservative protocols, as an expansion of the number of patients benefitting from transplantation can only be justified if the overall results of liver transplantation for HCC are maintained.
Adjuvant post-transplant treatment with sirolimus

Adjuvant treatments are commonly used after surgery for cancer to prevent recurrence and improve survival. Unlike other cancers, adjuvant management has not been taken into account after transplantation for HCC. While classical adjuvant treatments are based on combinations of chemotherapy drugs, post-transplant treatment should start with the use of immunosuppressive drug including anti-cancer properties, like sirolimus.

Sirolimus has been used in Edmonton since 1996. In a first study, described in Chapter 6, we assessed 70 patients with HCC (mean age: 54.4 ± 7, female/male: 12/58). Immunosuppression included de novo sirolimus, low dose calcineurin inhibitor for 6-12 months, with short-course (3 months) or no steroids. After 49 months-median follow-up, 8 patients have experienced an HCC recurrence, 2/34 when Milan criteria were respected (6%) and 6/36 when beyond Milan criteria (17%). One and four-year tumor-free survivals, were 85 and 73%, when Milan criteria were respected and 82% and 75% when they were not (p: 0.9). Incisional hernia (24/70, 34%), wound infection (12/70, 17%), anemia (39/70, 56%), leucopenia (39/70, 56%), high triglyceride (43/70, 61%) and cholesterol (28/70, 40%) levels and mouth ulcers (20/70, 29%) were among the most frequent complications. No hepatic artery thrombosis was observed.

Overall, these data suggest that de novo sirolimus-based immunosuppression is associated with satisfactory outcomes following transplantation, even in selected patients beyond Milan criteria. The protocol has proven safe, with the exception
of incisional hernia, significantly more frequent than in the non-sirolimus treated patients, and which speaks for a delayed (2-4 weeks) introduction of sirolimus.

A further validation of this study was conducted in Chapter 7, aiming at defining the imunosuppression associated with the best survival after liver transplantation for hepatocellular carcinoma (HCC). It was based on the Scientific Registry of Transplant Recipients and included 2491 adult recipients of isolated liver transplantation for HCC and 12167 for non-HCC diagnoses between March 2002 and March 2009. All patients remained on stable maintenance immunosuppression protocols for at least 6 months post-transplant. In a multivariate analysis, only anti-CD25 antibody induction and sirolimus-based maintenance therapy were associated with improved survivals after transplantation for HCC (HR 0.64, 95%CI 0.45-0.9, p ≤0.01; HR 0.53, 95%CI 0.31-0.92, p ≤0.05, respectively). The other studied drugs, including calcineurin-inhibitors, did not demonstrate a significant impact. In an effort to understand whether the observed effects were due to a direct impact of the drug on tumor or more on liver transplant in general, we conducted a similar analysis on non-HCC patients. While anti-CD25 induction was again associated with a trend toward improved survival, sirolimus showed a trend toward lower rates of survival in non-HCC recipients, confirming the specificity of its beneficial impact to cancer patients.
According to these data, sirolimus-based immunosuppression has unique post-transplant effects on HCC patients that lead to improved survival. Sirolimus should be more broadly used in case of transplantation for HCC.
Future developments

The future management of HCC will likely be more aggressive at all stages (Figure 9.4). Better screening policies will hopefully help identifying HCCs earlier and increase the proportion of patients benefitting from resection or transplantation. Listing in view of transplantation will also likely be possible in patients with more advanced HCCs. This change should however be performed step-by-step, in a conservative manner, in order to ensure that good outcomes are maintained. Such an expanded patient selection will likely be a mix of more permissive inclusion criteria and downstaging with a minimum waiting time of 3-6 months. From my point of view, it appears critical that such criteria include both morphological and biological variables, and I would favor the combination TTV ($\leq 115 \text{ cm}^3$) and AFP ($\leq 400 \text{ ng/ml}$). While on the waiting list, a more aggressive and continuous local HCC treatment appears justified, in order to decrease drop-out and improve survival.

The selection strategies will not only need to be implemented step-by-step, but they also require a continuous reassessment. To illustrate, improved post-transplant adjuvant management will likely lead to better post-transplant outcomes. As a consequence, improved outcomes could lead to the selection of patients with more advanced HCCs for transplantation and with maintained intent-to-treat survivals. While the use of sirolimus is one possible treatment, other drugs will likely be investigated both for the prophylaxis or the treatment of post-transplant recurrence.
Several key steps still need to be achieved:

- Expanded selection criteria: to be broadly accepted (I favor TTV≤115 cm³/AFP≤400 ng/ml)
- Downstaging: define downstaging selection criteria (I favor TTV≤250 cm³), minimum waiting time after downstaging and transplant criteria after downstaging
- Neo-adjuvant pre-transplant local HCC treatment: to be intensified
- Adjuvant post-transplant HCC treatment: broader use of sirolimus (acceptance by FDA), search for alternative compounds
- Treatment of recurrence: to be better studied and improved

With this road map in head, the management of HCC patients will keep on improving in a safe, efficient and fair manner.
References


